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(54) Title: DEAZAPURINE NUCLEOSIDE ANALOGS AND THEIR USE AS THERAPEUTIC AGENTS

(57) Abstract: Methods, compositions, and uses for various nucleoside analog libraries and library compounds are provided. Particularly preferred nucleosides include 6-C-purine nucleosides, 7,8-substituted purine nucleosides, pyrazolopyrimidine nucleoside analogs, various pyrimidine nucleosides, and triazine nucleosides, while preferred uses especially include use of such compounds as pharmacological, and particularly antiviral agents.

DEAZAPURINE NUCLEOSIDE ANALOGS AND THEIR USE AS THERAPEUTIC AGENTS

This application claims the benefit of U.S. provisional patent application with the serial number 60/350296, filed January 17, 2002, which is incorporated by reference herein.

Field of The Invention

The field of the invention is nucleoside analogs, and is especially directed towards various deazapurine nucleosides and their therapeutic use, particularly for treatment of viral infections with HCV, HRV, RSV, HIV, HBV, as well as viruses in the families of Flaviviridae, Paramyxoviridae, Orthomyxoviridae, Picornaviridae, Bunyaviridae, Arenaviridae, and Herpesviridae.

Background of The Invention

Nucleosides, and especially purine-type nucleosides and their analogs interact with many biological targets, and some nucleoside analogues have been used as antimetabolites for treatment of cancers and viral infections. After entry into the cell, many nucleoside analogues can be phosphorylated to monophosphates by nucleoside kinases, and then further phosphorylated by nucleoside monophosphate kinases and nucleoside diphosphate kinases to give nucleoside triphosphates. Once a nucleoside analogue is converted to its triphosphate inside the cell, it can be incorporated into DNA or RNA. Incorporation of certain unnatural nucleoside analogues into nucleic acid replicates or transcripts can interrupt gene expression by early chain termination or by interfering with the function of the modified nucleic acids. In addition, certain nucleoside analogue triphosphates are very potent, competitive inhibitors of DNA or RNA polymerases, which can significantly reduce the rate at which the natural nucleoside can be incorporated. Many anti-HIV nucleoside analogues fall into this category, including 3'-*C*-azido-3'-deoxythymidine, 2',3'-dideoxycytidine, 2',3'-dideoxyinosine, and 2',3'-didehydro-2',3'-dideoxythymidine.

Various purine-type and other nucleoside analogues can also act in other ways, for example, causing apoptosis of cancer cells and/or modulating immune systems. In addition to nucleoside antimetabolites, a number of nucleoside analogues that show very potent anticancer and antiviral activities act through still other mechanisms. Some well-known nucleoside anticancer drugs are thymidylate synthase inhibitors such as 5-fluorouridine, and

adenosine deaminase inhibitors such as 2-chloroadenosine. A well-studied anticancer compound, neplanocin A, is an inhibitor of S-adenosylhomocysteine hydrolase, which shows potent anticancer and antiviral activities.

Unfortunately, many nucleoside analogues that can inhibit tumor growth or viral infections are also toxic to normal mammalian cells, primarily because these nucleoside analogues lack adequate selectivity between the normal cells and the virus-infected host cells or cancer cells. For this reason, many otherwise promising nucleoside analogues fail to become therapeutics in the treatment of various diseases.

Selective inhibition of cancer cells or host cells infected by viruses has been an important subject for some time, and tremendous efforts have been made to search for more selective nucleoside analogues. In general, however, a large pool of nucleoside analogues is thought to be necessary in order to identify highly selective nucleoside analogues. Unfortunately, the classical method of synthesizing nucleosides and nucleotides having desired physiochemical properties, and then screening them individually, takes a significant amount of time to identify a lead molecule. Although thousands of nucleoside analogues were synthesized over the past decades, if both sugar and base modifications are considered, many additional analogues are still waiting to be synthesized.

During the last few years, combinatorial chemistry has been used to generate huge numbers of organic compounds other than nucleosides, nucleotides, and their analogs resulting in large compound libraries. If nucleosides, nucleotides, and their analogs could be made through a combinatorial chemistry approach, a large number of such compounds could be synthesized within months instead of decades and large libraries could be developed. A combinatorial chemistry approach to nucleosides may also encourage a focus beyond previously addressed biological targets. For example, in the past nucleoside analogues were usually designed as potential inhibitors of DNA or RNA polymerases and several other enzymes and receptors, including inosine monophosphate dehydrogenase, protein kinases, and adenosine receptors. If a vast number of diversified nucleoside analogues could be created, their uses may be far beyond those previously recognized biological targets, which would open a new era for the use of nucleoside analogues as human therapeutics.

The generation of combinatorial libraries of chemical compounds other than nucleosides, nucleotides, and their analogs by employing solid phase synthesis is well known

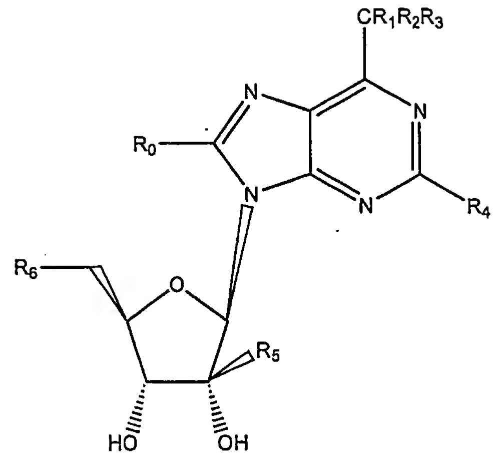
in the art. For example, Geysen, et al. (*Proc. Natl. Acac. Sci. USA*, 3998 (1984)) describes the construction of a multi-amino acid peptide library; Houghton, et al. (*Nature*, 354, 84 (1991)) describes the generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery; Lam, et al. (*Nature*, 354, 82 (1991)) describes a method of synthesis of linear peptides on a solid support such as polystyrene or polyacrylamide resin. Although a combinatorial chemistry approach has been proven to work well with many types of compounds, there are numerous problems with the generation of nucleoside libraries. Among numerous other difficulties, most nucleoside analogues contain a sugar moiety and a nucleoside base, which are linked together through a glycosidic bond. The formation of the glycosidic bond can be achieved through a few types of condensation reactions. However, most of the reactions do not give a very good yield of desired products, which may not be suitable to the generation of nucleoside libraries.

Moreover, the glycosidic bonds in many nucleosides are in labile to acidic condition, and many useful reactions in combinatorial chemistry approaches cannot be used in the generation of nucleoside analogue libraries. As a result, many researchers have focused their attention to areas in pharmaceutical chemistry that appear to present an easier access to potential therapeutic molecules, and there seems to be a lack of methods for generating libraries of nucleosides and nucleotides using solid phase synthesis. Therefore, there is still a need to provide new nucleoside compounds and methods for generation of nucleoside and nucleotide libraries.

<u>Summary of the Invention</u>

The present invention is directed to nucleoside analog libraries, library compounds, and their methods of use. Particularly contemplated nucleoside analog libraries will include library compounds with a modified sugar portion (most preferably modified at the C2'-position) and/or a modified purine base.

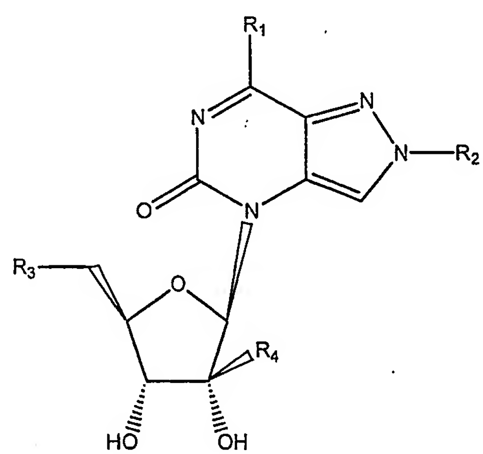
Thus, in one aspect of the inventive subject matter, contemplated compounds may have a structure according to **Formula 1**



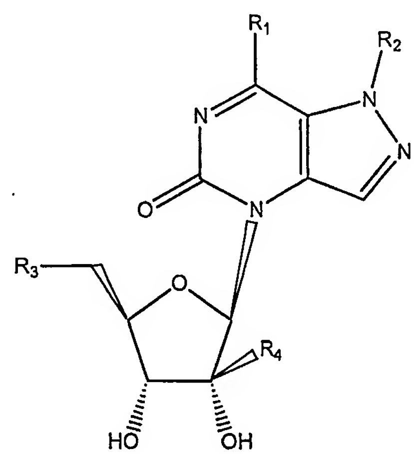
Formula 1

wherein the sugar is in D- or L-configuration; R_0 is H, halogen, alkyl, alkenyl, alkynyl, or aryl (all of which may be substituted); R_1, R_2, and R_3 are independently alkyl, alkenyl, alkynyl, aryl (all of which may be substituted), or H; and further preferred compounds include those in which R_1 and R_2 are H, and in which R_3 is alkyl-NR'R'', alkyl-ONR'R'', alkyl-NR'NR'R'', alkyl-SR', alkyl-OR', or alkyl-CN; R_4 is H or NH_2; R_5 is alkyl, alkenyl, alkynyl, aryl (all of which may be further substituted), CN, or CF_3, and most preferably methyl; R_6 is H, OH, phosphate, phosphonate, or boranophosphate; and R' and R'' are independently H, OH, or alkyl, alkenyl, alkynyl, aryl (all of which may be substituted).

In a further aspect of the inventive subject matter, contemplated compounds may have a structure according to **Formula 2A** or **Formula 2B**



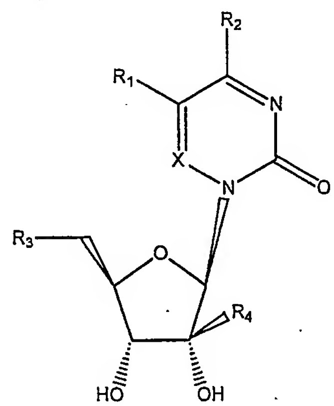
Formula 2A



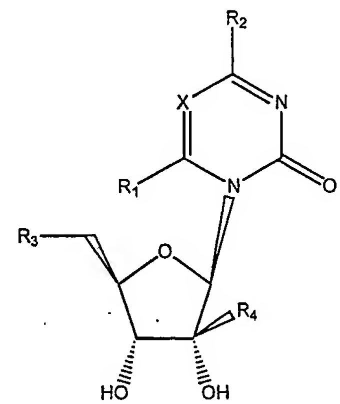
Formula 2B

wherein the sugar is in D- or L-configuration; R_1 is NR'R'', ONR'R'', NR'NR'R'', SR', OR', or R'; R_2 is alkyl, acyl, or aryl; R_3 is protected or unprotected OH, phosphate, phosphonate, or boranophosphate; R_4 is alkyl, alkenyl, alkynyl, aryl (all of which may be further substituted), CN, or CF_3, and most preferably methyl; and R' and R'' are independently H, OH, or alkyl, alkenyl, alkynyl, aryl (all of which may be substituted).

In a still further aspect of the inventive subject matter, contemplated compounds may have a structure according to **Formula 3A** or **Formula 3B**



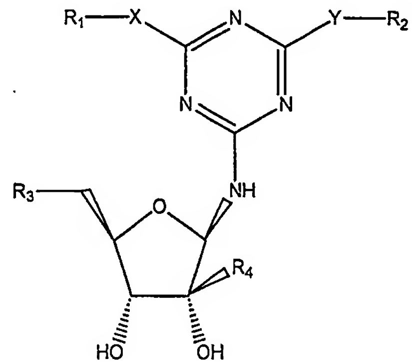
Formula 3A



Formula 3B

wherein the sugar is in D- or L-configuration; X is N or CH; R_1 is H, halogen, alkyl, or alkenyl; R_2 is NR'R'', ONR'R'', NR'NR'R'', SR', OR', or R'; R_3 is protected or unprotected OH, phosphate, phosphonate, or boranophosphate; R_4 is alkyl, alkenyl, alkynyl, aryl (all of which may be further substituted), CN, or CF_3, and most preferably methyl; and R' and R'' are independently H, OH, or alkyl, alkenyl, alkynyl, aryl (all of which may be substituted).

In yet another aspect of the inventive subject matter, contemplated compounds may have a structure according to **Formula 4**

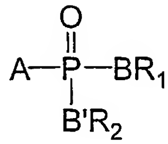


Formula 4

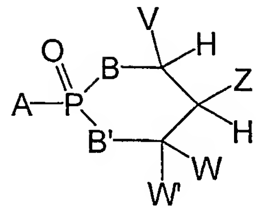
wherein X and Y are independently null, NR', O or S; R_1 and R_2 are independently NR'R", H, alkyl, alkenyl, alkynyl, or aryl (all of which may be further substituted); R_3 is protected or unprotected OH, phosphate, phosphonate, or boranophosphate; R_4 is alkyl, alkenyl, alkynyl, aryl (all of which may be further substituted), CN, or CF_3, and most preferably methyl; and R' and R" are independently H, OH, or alkyl, alkenyl, alkynyl, aryl (all of which may be substituted).

Further contemplated compounds also include phosphorylated forms (preferably at the 5'-atom), prodrugs, and/or metabolites of the above compounds, and in especially preferred aspects, such compounds may include a moiety (*e.g.*, a cyclic phosphate, a cyclic phosphonate, a cyclic phosphoamidate, or a non-cyclic phosphate (di-) ester) that is covalently coupled to the C2'-atom, C3'-atom, and/or C5'-atom (thereby replacing the corresponding OH group), wherein at least part of the moiety may be preferentially cleaved from the compound in a target cell or target organ.

Therefore, in a further aspect of the inventive subject matter, preferred moieties will have a structure according to **Formulae M1** or **M2**, wherein A, B, B', V, W, W', and Z are defined as in the section entitled "Contemplated Compounds" below. Yet further contemplated prodrugs include SATE (S-acyl-thio-ethyl) and pivalic acid ester-prodrug forms of contemplated compounds.



M1



M2

In a still further aspect of the inventive subject matter, a pharmaceutical composition includes contemplated compounds at a concentration effective to reduce viral propagation of a virus in a patient infected with the virus (*e.g.*, HCV virus, an HRV virus, an RSV virus, an HIV virus, and an HBV virus). Contemplated compositions may further comprise a second pharmacologically active molecule, and particularly preferred molecules include a cytokine (and fragments thereof), immunomodulators, and antibodies.

Consequently, the inventors contemplate a method of treating a viral infection in a patient in which contemplated compounds are administered to the patient in an amount effective to reduce viral propagation. Viewed from another perspective, the inventors contemplate a method of reducing viral propagation in a cell infected with a virus, wherein contemplated compounds present the cell in an amount effective to reduce viral propagation.

Various objects, features, aspects and advantages of the present invention will become more apparent from the following detailed description of preferred embodiments of the invention.

Detailed Description

The inventors have discovered that various nucleoside, and especially various deazapurine nucleoside analogs, may be employed as therapeutic molecules, and especially as antiviral agents (*e.g.*, against HCV).

The term "nucleoside library" as used herein refers to a plurality of chemically distinct nucleosides, nucleotides, nucleoside analogs, and/or nucleotide analogs wherein at least some of the nucleosides, nucleotides, nucleoside analogs, and/or nucleotide analogs include, or have been synthesized from a common precursor.

For example, a plurality of nucleosides, nucleotides, nucleoside analogs, and/or nucleotide analogs that were prepared from a protected ribofuranose as a building block/precursor is considered a nucleoside library under the scope of this definition. Therefore, the term "common precursor" may encompass a starting material in a first step in a synthesis as well as a synthesis intermediate (*i.e.*, a compound derived from a starting material). In another example, at least one step in the synthesis of one of the nucleosides, nucleotides, nucleoside analogs, and/or nucleotide analogs is concurrent with at least one step in the synthesis of another one of the nucleosides, nucleotides, nucleoside analogs, and/or nucleotide analogs, and synthesis is preferably at least partially automated. In contrast, a collection of individually synthesized nucleosides, nucleotides, nucleoside analogs, and/or nucleotide analogs, and especially a collection of compounds not obtained from a nucleoside library, is not considered a nucleoside library because such nucleosides, nucleotides, nucleoside analogs, and/or nucleotide analogs will not have a common precursor, and because such nucleosides, nucleotides, nucleoside analogs, and/or nucleotide analogs are not concurrently produced.

It is further generally contemplated that the complexity of contemplated libraries is at least 20 distinct nucleosides, nucleotide, nucleoside analogs, and/or nucleotide analogs, more typically at least 100 distinct nucleosides, nucleotide, nucleoside analogs, and/or nucleotide analogs, and most typically at least 1,000 distinct nucleosides, nucleotide, nucleoside analogs, and/or nucleotide analogs. Consequently, a typical format of a nucleoside library will include multi-well plates or a plurality of small volume (*i.e.*, less than 1 ml) vessels coupled to each other. The term "library compound" as used herein refers to a nucleoside, nucleotide, nucleoside analog, and/or nucleotide analog within a nucleoside library.

The term "nucleoside" refers to all compounds in which a heterocyclic base is covalently coupled to a sugar, and an especially preferred coupling of the nucleoside to the sugar includes a C1'-(glycosidic) bond of a carbon atom in a sugar to a carbon or heteroatom (typically nitrogen) in the heterocyclic base. The term "nucleoside analog" as used herein refers to all nucleosides in which the sugar is not a ribofuranose and/or in which the heterocyclic base is not a naturally occurring base (*e.g.,* A, G, C, T, I, etc.). It should further be particularly appreciated that the terms nucleoside and nucleoside analog also include all prodrug forms of a nucleoside or nucleoside analog, wherein the prodrug form may be activated/converted to the active drug/nucleoside in one or more than one step, and wherein

the activation/conversion of the prodrug into the active drug/nucleoside may occur intracellularly or extracellularly (in a single step or multiple steps). Especially contemplated prodrug forms include those that confer a particular specificity towards a diseased or infected cell or organ, and exemplary contemplated prodrug forms are described in "Prodrugs" by Kenneth B. Sloan (Marcel Dekker; ISBN: 0824786297), "Design of Prodrugs" by Hans Bundgaard (ASIN: 044480675X), or in copending US application number 09/594410, filed 06/16/2000, all of which are incorporated by reference herein.

Similarly, the term "nucleotide" as used herein refers to a nucleoside that is coupled to a 5'-phosphate group (or modified phosphate group, including phosphonate, thiophosphate, phosphate ester, etc.). Consequently, the term "nucleotide analog" refers to a nucleoside analog that is coupled to a 5'-phosphate group (or modified phosphate group, including phosphonate, thiophosphate, phosphate ester, etc.).

As used herein, the terms "heterocycle" and "heterocyclic base" are used interchangeably herein and refer to any compound in which a plurality of atoms form a ring via a plurality of covalent bonds, and wherein the ring includes at least one atom other than a carbon atom. Particularly contemplated heterocyclic bases include 5- and 6-membered rings with nitrogen, sulfur, or oxygen as the non-carbon atom (*e.g.*, imidazole, pyrrole, triazole, dihydropyrimidine). Further contemplated heterocylces may be fused (*i.e.*, covalently bound) to another ring or heterocycle, and are thus termed "fused heterocycle" as used herein. Especially contemplated fused heterocycles include a 5-membered ring fused to a 6-membered ring (*e.g.*, purine, 7-deazapurine, 7-deaza-8-azapurine, 3-deazapurine, or 9-deazapurine).

Still further contemplated heterocyclic bases may be aromatic, or may include one or more double or triple bonds. Moreover, contemplated heterocyclic bases may further include one or more substituents other than hydrogen, and especially contemplated substituents include those referenced below. Contemplated heterocycles or substituted heterocycles are typically attached directly to nucleoside bases or sugars, but coupling of the heterocyclic base to the sugar may also include a linker moiety with at least 1-4 atoms between the heterocyclic base and the sugar.

As further used herein, the term "sugar" refers to all carbohydrates and derivatives thereof, wherein particularly contemplated derivatives include deletion, substitution or

addition of a chemical group in the sugar. For example, especially contemplated deletions include 2'-deoxy and/or 3'-deoxy sugars. Especially contemplated substitutions include replacement of the ring-oxygen with sulfur, methylene, or nitrogen, or replacement of a hydroxyl group with a halogen, an amino-, sulfhydryl-, or methyl group, and especially contemplated additions include methylene phosphonate groups, 2'-beta-methyl and/or 3'-beta-methyl groups. Further contemplated sugars also include sugar analogs (*i.e.*, not naturally occurring sugars), and particularly carbocyclic ring systems. The term " carbocyclic ring system" as used herein refers to any molecule in which a plurality of carbon atoms form a ring, and in especially contemplated carbocyclic ring systems the ring is formed from 3, 4, 5, or 6 carbon atoms. Examples of these and further preferred sugars are provided below.

The terms "alkyl" and "unsubstituted alkyl" are used interchangeably herein and refer to any linear, branched, or cyclic hydrocarbon in which all carbon-carbon bonds are single bonds. The term "substituted alkyl" as used herein refers to any alkyl that further comprises a functional group, and particularly contemplated functional groups include nucleophilic (*e.g.*, $-NH_2$, -OH, -SH, -NC, etc.) and electrophilic groups (*e.g.*, C(O)OR, C(X)OH, etc.), polar groups (*e.g.*, -OH), non-polar groups (*e.g.*, aryl, alkyl, alkenyl, alkynyl, etc.), ionic groups (e.g., $-NH_3^+$), and halogens (*e.g.*, -F, -Cl), and all chemically reasonable combinations thereof. The terms "alkenyl" and "unsubstituted alkenyl" are used interchangeably herein and refer to any linear, branched, or cyclic alkyl with at least one carbon-carbon double bond. The term "substituted alkenyl" as used herein refers to any alkenyl that further comprises a functional group, and particularly contemplated functional groups include those discussed above.

Furthermore, the terms "alkynyl" and "unsubstituted alkynyl" are used interchangeably herein and refer to any linear, branched, or cyclic alkyl or alkenyl with at least one carbon-carbon triple bond. The term "substituted alkynyl" as used herein refers to any alkynyl that further comprises a functional group, and particularly contemplated functional groups include those discussed above. The terms "aryl" and "unsubstituted aryl" are used interchangeably herein and refer to any aromatic cyclic, alkenyl, or alkynyl. The term "substituted aryl" as used herein refers to any aryl that further comprises a functional group, and particularly contemplated functional groups include those discussed above. The term "alkaryl" is employed where the aryl is further covalently bound to an alkyl, alkenyl, or alkynyl.

Thus, the term "substituted" as used herein also refers to a replacement of a chemical group or substituent (typically H or OH) with a functional group, and particularly contemplated functional groups include nucleophilic (*e.g.*, $-NH_2$, -OH, -SH, -NC, etc.) and electrophilic groups (*e.g.*, C(O)OR, C(X)OH, etc.), polar groups (*e.g.*, -OH), non-polar groups (*e.g.*, aryl, alkyl, alkenyl, alkynyl, etc.), ionic groups (*e.g.*, $-NH_3^+$), and halogens (*e.g.*, -F, -Cl), and all chemically reasonable combinations thereof.

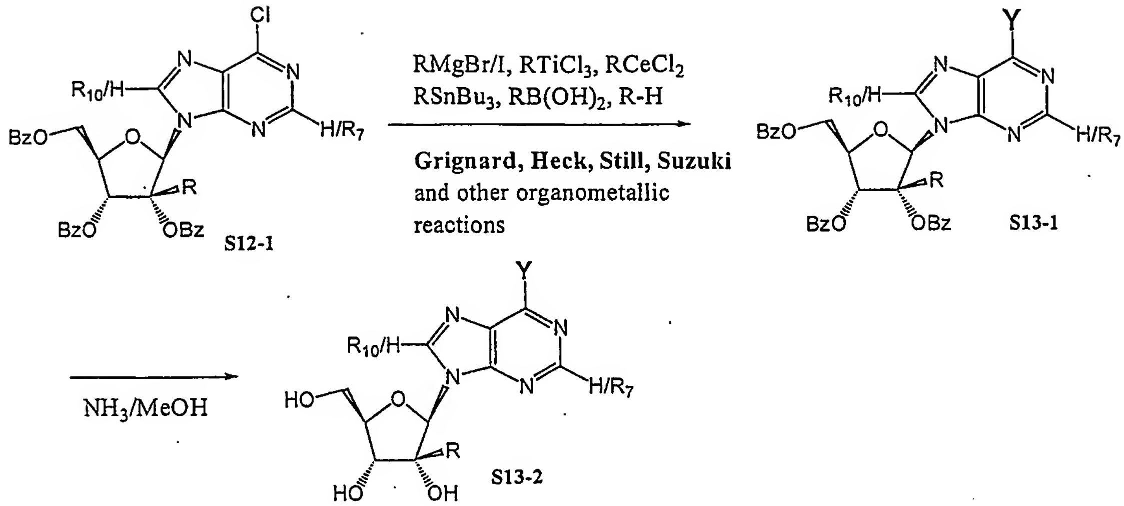
Contemplated Nucleosides

The present invention is generally directed to various heterocyclic nucleoside libraries and library compounds within these libraries, wherein contemplated compounds may be synthesized by medicinal and combinatorial approaches using solution and/or solid phase strategies. Furthermore, while most of the schemes below depict nucleosides and nucleoside analogs, it should be recognized that all of the contemplated nucleosides and nucleoside analogs may also be phosphorylated (preferably at the C5'-position) to the corresponding nucleotide or nucleotide analogs. Moreover, it should be appreciated that all prodrug forms and metabolites of the compounds according to the inventive subject matter presented herein are also contemplated.

6-C-Substituted Purine Nucleoside Libraries and Compounds

The inventors discovered that a 6-C-substituted purine nucleoside analog library and library compounds may be prepared by reacting an appropriate nucleoside having a leaving group in the 6-position with a strong nucleophilic reagent, which may or may not include coupling of the nucleoside to a solid phase.

More particularly, in one exemplary approach depicted in **Scheme 1**, a modified sugar is prepared by protecting the OH groups of the sugar, and subsequent selective deprotection and oxidation at the 2'-position via Dess-Martin reagent. The so prepared 2'-oxo-sugar is then reacted with a 2'-modifying reagent (*e.g.*, Grignard reagent) to yield the corresponding 2'-modified sugar that is then covalently coupled to a selected heterocyclic base with a leaving group in the 6-position. The resulting 2'-modified nucleoside analog is then reacted in a Grignard, Heck, Stille, or Suzuki reaction to replace the leaving group with a substituent, wherein the substituent is coupled to the 6-position via a carbon atom.



R_1 = H, CH_3, $CH{=}CH_2$, CH_2CH_3, $CH_2CH{=}CH_2$, -cyclopropyl, cyclobutyl, $CH(CH_3)_2$, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, CHF_2, CF_3, $CHCl_2$, CCl_3, CN, $C{\equiv}CH$ and others as described in different sections

R_7, R_{10} as described previously

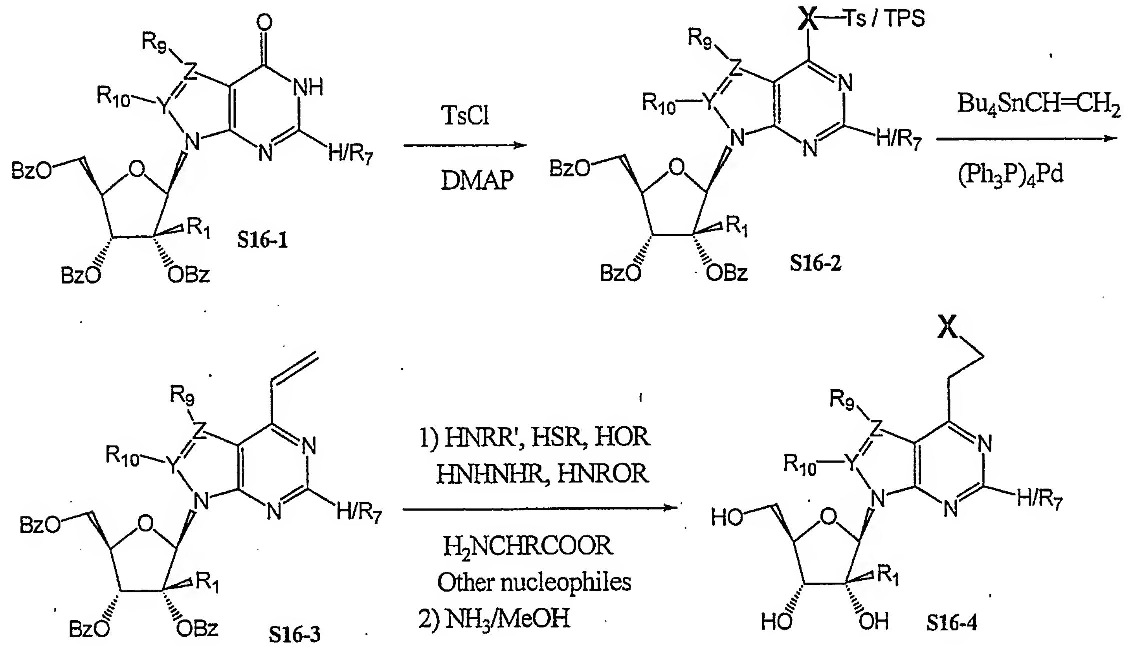
Y = alkyl, akenyl, alkynyl, aryl, substituted alkyl/alkenyl/alkynyl/aryl, heterocycles and other substituents in the building block lists

Scheme 1

It should be especially recognized that numerous 2'-modified sugar portions may be prepared according to the inventive subject matter, and that the chemical and biological nature of particular 2-modified sugars will typically depend on the modification reagent employed. Thus, all known Heck, Suzuki, Grignard, and Stille reagents are contemplated suitable for use herein, and a collection of exemplary suitable Heck, Suzuki, and Stille reagents is provided in the section entitled "Experiments and Data" below.

Similarly, the nature of the heterocyclic base may vary considerably, and all purine bases are considered suitable for use herein so long as such heterocyclic bases include a leaving group in the 6-position. Consequently, contemplated heterocyclic purine bases may be substituted in the 8-position and/or the 2-position with various substituents. Exemplary substituents for the 8-position include H, halogen, alkyl, alkenyl, alkynyl, or aryl (all of which may optionally be substituted) and exemplary substituents for 2-position may vary as well, but especially preferred substituents include H and NH_2. Furthermore, it should be recognized that all or almost all of such alternative heterocyclic bases are commercially available, or may be prepared by a person of ordinary skill in the art following well established protocols.

Alternatively, and especially where the newly introduced substituent in the 6-position has a reactive group (*e.g.*, double bond) that can be further modified, an exemplary synthetic route as shown in **Scheme 2** may be employed. Here, an ethenyl group is introduced into the 6-position of a nucleoside that was prepared following similar procedures as described above. The so introduced ethenyl group is then employed as an electrophilic group that is further modified with a nucleophilic group.



R_1 = H, CH_3, $CH{=}CH_2$, CH_2CH_3, $CH_2CH{=}CH_2$, -cyclopropyl, cyclobutyl, $CH(CH_3)_2$, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, CHF_2, CF_3, $CHCl_2$, CCl_3, CN, $C{\equiv}CH$ and others as described in different sections

R_7, R_{10}, Z, Y are as described previously

X = NH_2, NHR, NRR, NHOH, NHOR, ONH_2, ONHR, $NHNH_2$, NHNHR, NRNHR, NHNRR, NRNRR, OR, SR, N_3, CN, NHCHRCOOR, amino acids (R are alkyl, akenyl, alkynyl, aryl, heterocycles and others) as well as other substituents in the building block lists

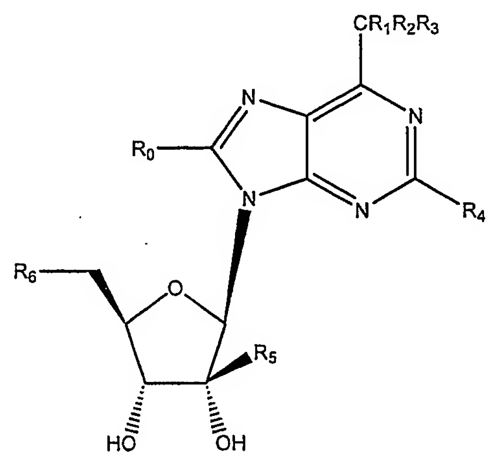
Scheme 2

With respect to the sugar, sugar modification, and the heterocyclic base, the same considerations as above apply. With further respect to the heterocyclic base, it is contemplated that the nature of suitable heterocyclic bases may vary considerably, and all purine bases are considered appropriate so long as such heterocyclic bases include an oxo-group in the 6-position. Consequently, contemplated heterocyclic purine bases may also be

substituted in the 8-position and/or the 2-position with various substituents. Exemplary substituents for the 8-position include H, halogen, alkyl, alkenyl, alkynyl, or aryl (all of which may optionally be substituted) and exemplary substituents for the 2-position may vary as well, but especially preferred substituents include H and NH_2. Furthermore, it should be recognized that all or almost all of such alternative heterocyclic bases are commercially available, or may be prepared by a person of ordinary skill in the art following well established protocols.

The inventors still further contemplate that modification of the introduced 6-substituent may provide a plurality of potential nucleoside analogs, and it should be recognized that the particular nature of the modification will typically depend on the type of reagent employed. However, it is generally preferred that the reagent for further modification is a nucleophilic reagent, and especially preferred nucleophilic reagents include a primary and/or secondary amine.

Therefore, contemplated library compounds will include those having a general structure according to Formula 1



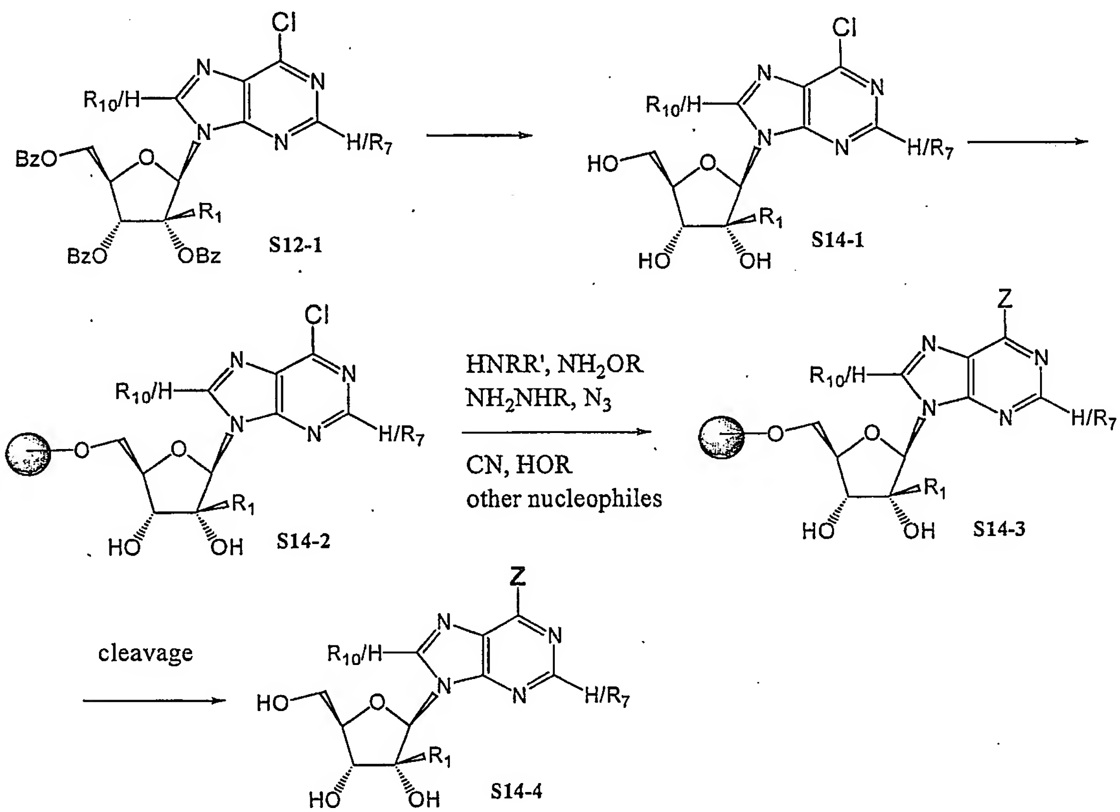
Formula 1

wherein the sugar is in D- or L-configuration; R_0 is H, halogen, or alkyl, alkenyl, alkynyl, aryl (all of which may be substituted); R_1, R_2, and R_3 are independently alkyl, alkenyl, alkynyl, aryl (all of which may be substituted), or H, and further preferred compounds include those in which R_1 and R_2 are H, and in which R_3 is alkyl-NR'R", alkyl-ONR'R", alkyl-NR'NR'R", alkyl-SR', alkyl-OR', or alkyl-CN; R_4 is H or NH_2; R_5 is alkyl,

alkenyl, alkynyl, aryl (all of which may be further substituted), CN, or CF_3, and most preferably methyl; R_6 is H, OH, phosphate, phosphonate, or boranophosphate; and R' and R" are independently H, OH, or alkyl, alkenyl, alkynyl, aryl (all of which may be substituted).

<u>C$_2$'-Substituted-6,8-Modified Purine Nucleoside Libraries</u>

In another contemplated aspect of the inventive subject matter, the inventors discovered that purine nucleoside analogs with 6-N-substituents may be prepared in a manner substantially similar to the methods described above, wherein the nucleophilic reagent to be introduced into the 6-position is a nucleophilic reagent other than a Heck/Still/Suzuki, and/or Grignard reagent. Such particularly preferred reagents include primary and secondary amines, alcohols, thiols, etc., and an exemplary synthetic route is depicted in **Schemes 3** and **Scheme** 4 below.

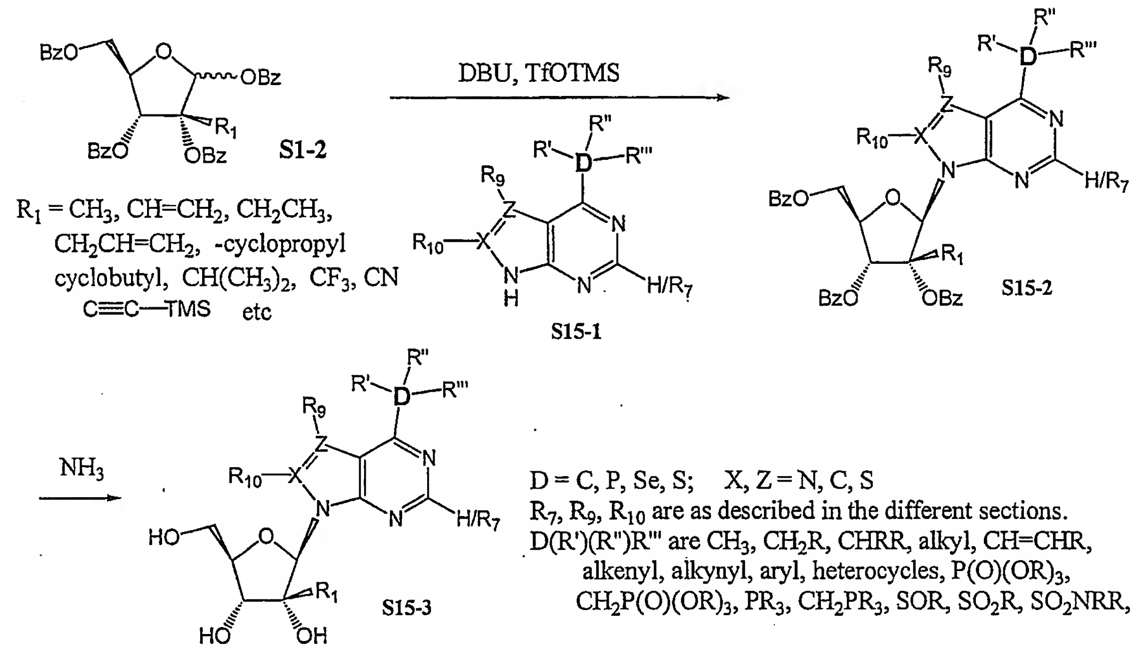


R_1 = H, CH_3, $CH=CH_2$, CH_2CH_3, $CH_2CH=CH_2$, -cyclopropyl, cyclobutyl, $CH(CH_3)_2$, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, CHF_2, CF_3, $CHCl_2$, CCl_3, CN, $C\equiv CH$ and others as described in different sections

R_7, R_{10} as described previously

X = NH_2, NHR, NRR, NHOH, NHOR, ONH_2, ONHR, $NHNH_2$, NHNHR, NRNHR, NHNRR, NRNRR, OR, SR, N_3, CN, etc (R are alkyl, akenyl, alkynyl, aryl, heterocycles and others) as well as other substituents in the building block lists

Scheme 3



R_1 = CH_3, CH=CH_2, CH_2CH_3, CH_2CH=CH_2, -cyclopropyl cyclobutyl, $CH(CH_3)_2$, CF_3, CN C≡C—TMS etc

D = C, P, Se, S; X, Z = N, C, S
R_7, R_9, R_{10} are as described in the different sections.
D(R')(R")R"' are CH_3, CH_2R, CHRR, alkyl, CH=CHR, alkenyl, alkynyl, aryl, heterocycles, $P(O)(OR)_3$, $CH_2P(O)(OR)_3$, PR_3, CH_2PR_3, SOR, SO_2R, SO_2NRR,

R_1 = H, CH_3, CH=CH_2, CH_2CH_3, CH_2CH=CH_2, -cyclopropyl, cyclobutyl, $CH(CH_3)_2$, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, CHF_2, CF_3, $CHCl_2$, CCl_3, CN, C≡CH and others as described in different sections
R_7, R_{10} as described previously

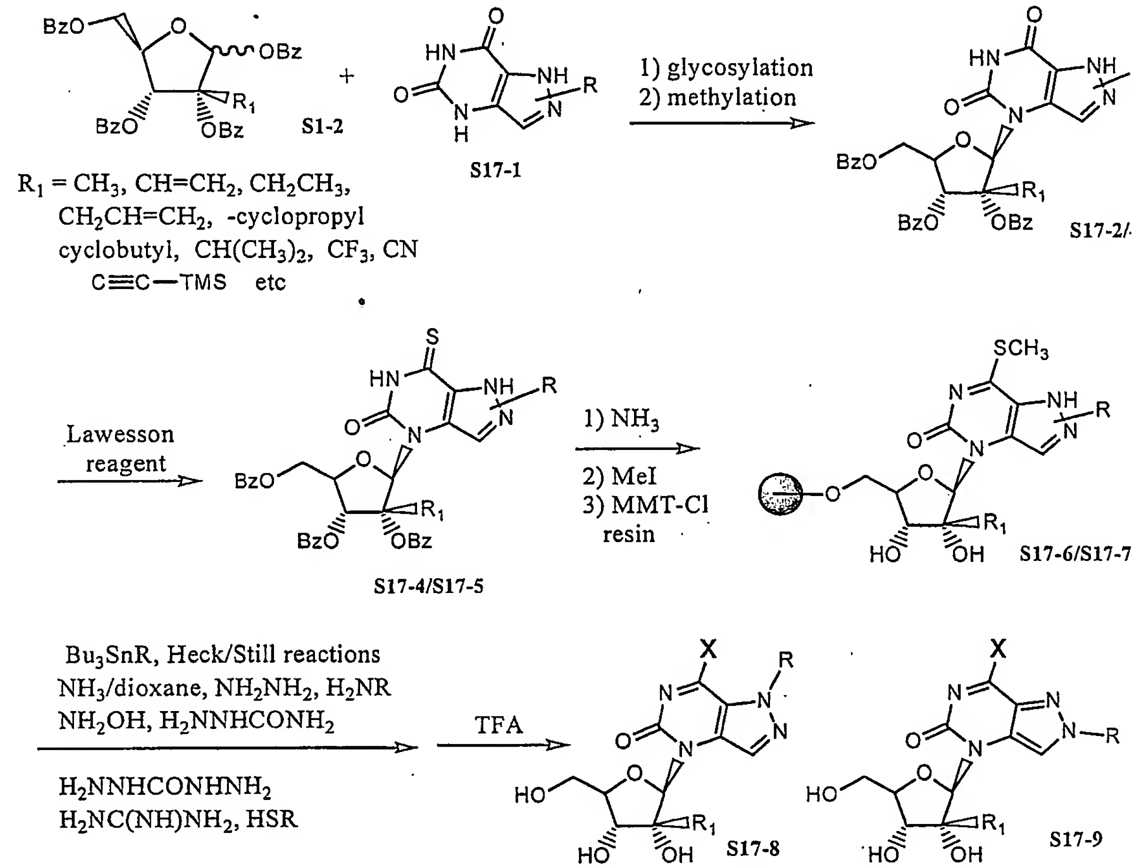
Scheme 4

Here, a suitably C_2'-substituted and protected sugar is first coupled to an optionally 7,8-modified purine heterocyclic base having a leaving group in the 6-position, which is then replaced by the nucleophilic reagent. Alternatively, a 2'-modified sugar is coupled to a 6-modified heterocyclic base to provide the desired nucleoside analog. With respect to the sugar, the sugar modification, and the heterocyclic base, the same considerations as described above apply.

C_2'-Substituted Modified Pyrazolopyrimidine Nucleoside Libraries

In a still further contemplated aspect of the inventive subject matter, the inventors discovered that C_2'-substituted modified pyrazolopyrimidine nucleoside libraries and compounds can be prepared. In an exemplary approach, as depicted in **Scheme 5** below, a heterocyclic base is coupled via the six-membered ring to a previously prepared 2'-modified sugar. In a further step or steps, one or both ketocarbonyl-groups are replaced with a thiocarbonyl group, wherein the thiocarbonyl group is further converted to a leaving group

that is subsequently replaced by a nucleophilic reagent to yield the desired compound(s). Of course it should be appreciated that one or more of the below depicted reactions may be performed in solution wherein the reagent or nucleoside analog is coupled to a solid phase.



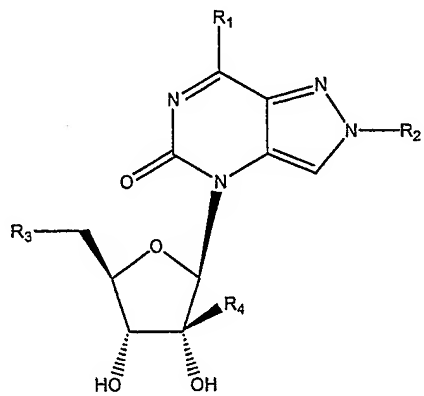
R_1 are the substituents described in different sections
R = alkyl, acyl, aryl, heterocycles
X = alkyl, aryl, alkenyl, alkynyl, NH_2, $NHNH_2$, NHR, NR_2, NHOH, NHOR, $NHNHCONH_2$, $NHNHCONHNH_2$, SR, CN, amidine, guanidine, hydroxyguanidine, mercaptoguanidine and other substituents in the building block lists

Scheme 5

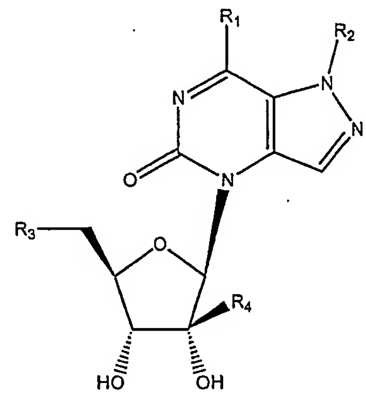
With respect to the sugar and the sugar modification, the same consideration as described above in the section entitled "6-C-Substituted Purine Nucleoside Libraries and Compounds" applies. It should further be recognized that various methods other than conversion of the ketocarbonyl group via a thiocarbonyl group to a leaving group may be employed, and all known alternative methods are contemplated suitable for use herein. For example, suitable conversions may employ reaction with TPSCl as described in Scheme 2 above.

It should still further be recognized that there are numerous reagents suitable for replacement of the leaving group in the heterocyclic base, and all known nucleophilic reagents are contemplated suitable for use herein. However, it is generally preferred that the nucleophilic reagent will include a primary and/or secondary amine, an alcohol, a thiol, a Heck-, Stille-, or Suzuki-reagent, or an organometallic reagent. Exemplary suitable nucleophilic reagents are provided in the section entitled "Experiments and Data" below.

Therefore, contemplated library compounds will include those having a general structure according to Formula 2A or Formula 2B.



Formula 2A

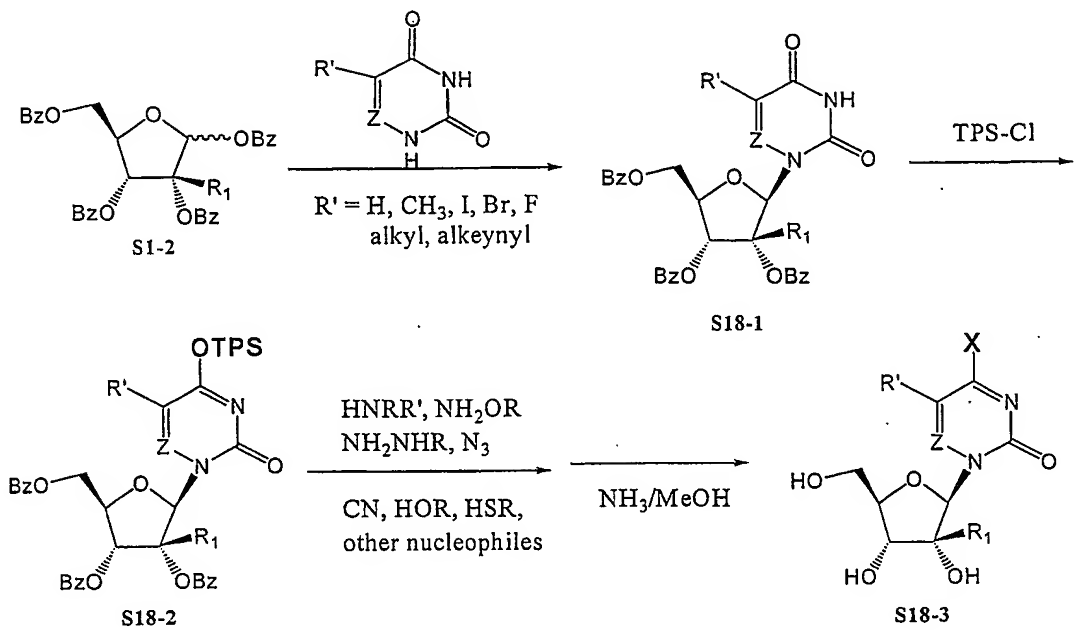


Formula 2B

wherein the sugar is in D- or L-configuration; R_1 is NR'R", ONR'R", NR'NR'R", SR', OR', or R'; R_2 is alkyl, acyl, or aryl; R_3 is protected or unprotected OH, phosphate, phosphonate, or boranophosphate; R_4 is alkyl, alkenyl, alkynyl, aryl (all of which may be further substituted), CN, or CF_3, and most preferably methyl; and R' and R" are independently H, OH, or alkyl, alkenyl, alkynyl, aryl (all of which may be substituted).

C_2'-Substituted-Pyrimidine Nucleoside Analog Libraries

In yet further contemplated aspects of the inventive subject matter, the inventors discovered that C_2'-substituted-pyrimidine nucleoside analog libraries and compounds may be prepared in a protocol in which a modified pyrimidine heterocyclic base is coupled to a suitably protected C_2'-substituted sugar. An exemplary route for such libraries and compounds is depicted in **Scheme 6**. The so prepared nucleoside is then further modified to the desired compound(s).



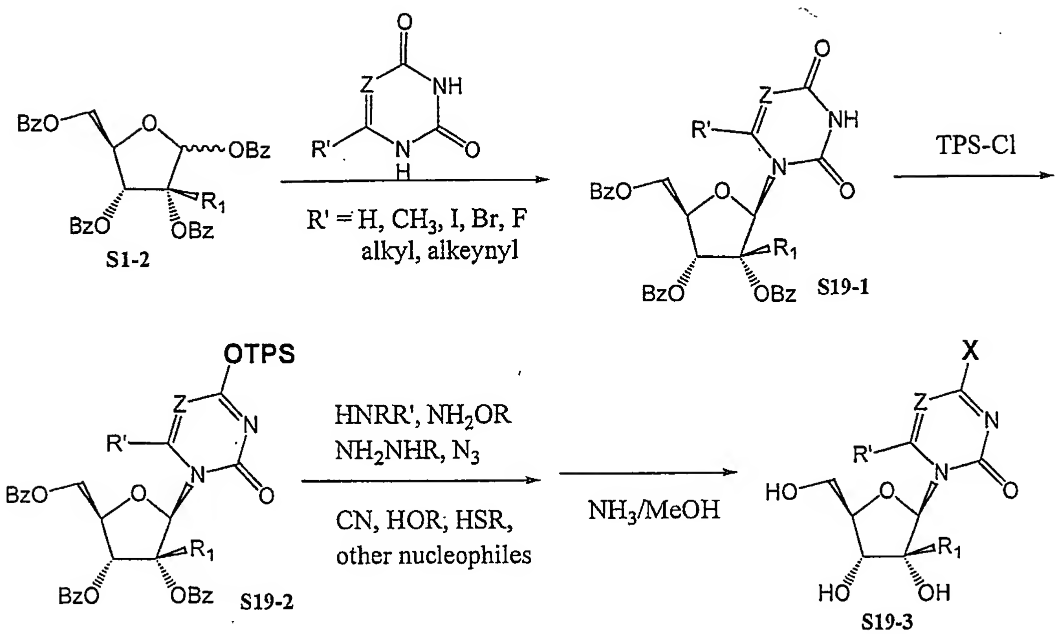
Z = N, CH, CR
R' = aryl, heterocycles, alkenyl, alkynyl, substituted alkenyl/alkynyl and other substituents in the building lists

R_1 = H, CH_3, $CH{=}CH_2$, CH_2CH_3, $CH_2CH{=}CH_2$, -cyclopropyl, cyclobutyl, $CH(CH_3)_2$, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, CHF_2, CF_3, $CHCl_2$, CCl_3, CN, $C{\equiv}CH$ and others as described in different sections

X = NH_2, NHR, NRR, NHOH, NHOR, ONH_2, ONHR, $NHNH_2$, NHNHR, NRNHR, NHNRR, NRNRR, OR, SR, N_3, CN, NHCHRCOOR, amino acids (R are alkyl, akenyl, alkynyl, aryl, heterocycles and others) as well as other substituents in the building block lists

Scheme 6

Alternatively, where it is desired that the radicals Z and R1 of the heterocyclic base are switched in their position (as compared to the heterocyclic base of Scheme 6), contemplated nucleoside analog libraries and compounds may be prepared as depicted in **Scheme** 7 below.



Z = N, CH, CR

R' = aryl, heterocycles, alkenyl, alkynyl, substituted alkenyl/alkynyl and other substituents in the building lists

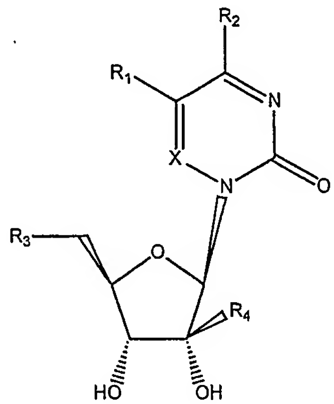
R_1 = H, CH_3, $CH{=}CH_2$, CH_2CH_3, $CH_2CH{=}CH_2$, -cyclopropyl, cyclobutyl, $CH(CH_3)_2$, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, CHF_2, CF_3, $CHCl_2$, CCl_3, CN, C≡CH and others as described in different sections

X = NH_2, NHR, NRR, NHOH, NHOR, ONH_2, ONHR, $NHNH_2$, NHNHR, NRNHR, NHNRR, NRNRR, OR, SR, N_3, CN, NHCHRCOOR, amino acids (R are alkyl, akenyl, alkynyl, aryl, heterocycles and others) as well as other substituents in the building block lists

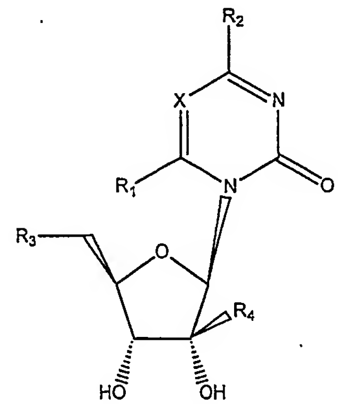
Scheme 7

With respect to the sugar, the sugar modification, and the coupling of the sugar to the heterocyclic base, the same considerations as discussed above apply. Furthermore, it should be appreciated that where the carbonyl oxygen can be converted into a leaving group using various methods well known in the art, suitable alternative methods to conversion with TPS-Cl include those as described in Scheme 5 above. Similarly, the nature of contemplated nucleophiles to replace the leaving group from the pyrimidine heterocyclic base may vary considerably, and all nucleophilic groups discussed above are considered suitable for use in conjunction with the teachings presented herein. Exemplary nucleophilic reagents therefore include various alcohols, thiols, organo-metallic reagents, and primary and secondary amines. Further particularly suitable exemplary nucleophilic reagents are listed in the section entitled "Experiments and Data" below.

Therefore, contemplated libraries and library compounds may include those having a structure according to **Formula 3A** or **Formula 3B**



Formula 3A

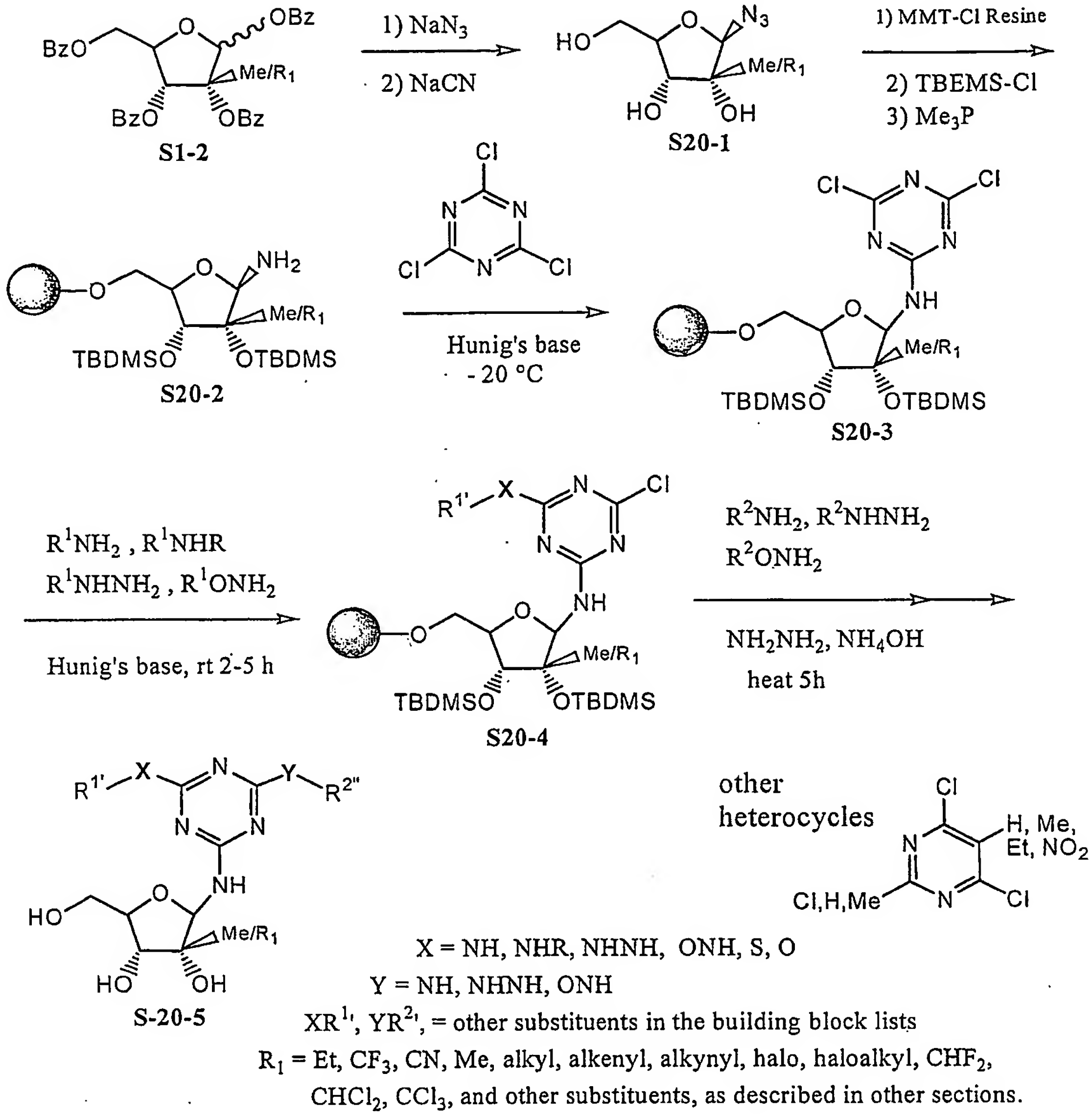


Formula 3B

wherein the sugar is in D- or L-configuration; X is N or CH; R_1 is H, halogen, alkyl, or alkenyl; R_2 is NR'R", ONR'R", NR'NR'R", SR', OR', or R'; R_3 is protected or unprotected OH, phosphate, phosphonate, or boranophosphate; R_4 is alkyl, alkenyl, alkynyl, aryl (all of which may be further substituted), CN, or CF_3, and most preferably methyl; and R' and R" are independently H, OH, or alkyl, alkenyl, alkynyl, aryl (all of which may be substituted).

<u>C_2'-Substituted-Exo-Triazine Nucleoside Libraries</u>

In yet another contemplated aspect of the inventive subject matter, the inventors discovered that C_2'-substituted-triazine nucleoside libraries and compounds may be synthesized as shown in **Scheme 8** below, wherein a triazine heterocyclic base is coupled to the C_2'-substituted sugar via an atom other than carbon (here: amino group). In one exemplary route, trichlorotriazine is coupled to a 1'-amino sugar to form the corresponding nucleoside which is then further modified to the desired compound(s).



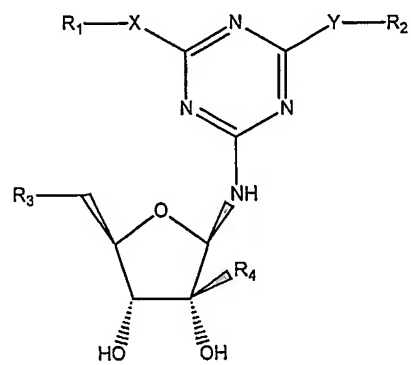
Scheme 8

Again, with respect to the sugar and the 2'-sugar modification, the same considerations as discussed above apply. Furthermore, it should be appreciated that while preparation of the amino sugar via an azido intermediate is generally preferred, commercially available 1'-amino sugars and their modifications are also considered suitable.

In a generally preferred aspect of the inventive subject matter, it is contemplated that the so prepared amino sugars may be coupled to a variety of heterocyclic bases, and it is generally contemplated that all heterocyclic bases are considered suitable so long as such bases (a) may be coupled via the NH_2 group of the sugar to the sugar, and (b) have at least one leaving group that can be replaced in a later step with a nucleophilic reagent. However, it

is particularly preferred that the heterocyclic base is a trichlorotriazine that is modified in a series of reactions to replace both remaining Cl- substituents with the respective nucleophilic reagents. Once more, with respect to the nucleophilic reagents that replace the leaving group(s) at the heterocyclic base, the same considerations as described above apply.

Therefore, contemplated libraries and library compounds may include those having a structure according to **Formula 4**



Formula 4

wherein X and Y are independently null, NR', O or S; R_1 and R_2 are independently NR'R'', H, alkyl, alkenyl, alkynyl, or aryl (all of which may be further substituted); R_3 is protected or unprotected OH, phosphate, phosphonate, or boranophosphate; R_4 is alkyl, alkenyl, alkynyl, aryl (all of which may be further substituted), CN, or CF_3, and most preferably methyl; and R' and R'' are independently H, OH, or alkyl, alkenyl, alkynyl, aryl (all of which may be substituted).

In particularly preferred aspects of the inventive subject matter, the sugar of above contemplated compounds is a ribofuranose in D- or L-configuration, which may in some aspects be further substituted in one or more positions. For example, where the nucleoside analog is employed as a substrate or cosubstrate for an enzyme using nucleotides, contemplated 6,7-disubstituted-7-deazapurine nucleosides may include a phosphate group (or phosphate analog, including phosphonate, phosphoamidate, or thiophosphate) coupled to the C5'-position. Depending on the particular location of the enzyme, the charge of the phosphate group may be masked by chemical modification to facilitate penetration of contemplated compounds across a cell membrane, and suitable modifications include esterification (*e.g.*, pivaloyl ester, or S-acyl-esters), amidation, ether formation, etc. In especially preferred

modifications, at least part of the modification is cleaved from the compound once the compound enters a cell (*infra*).

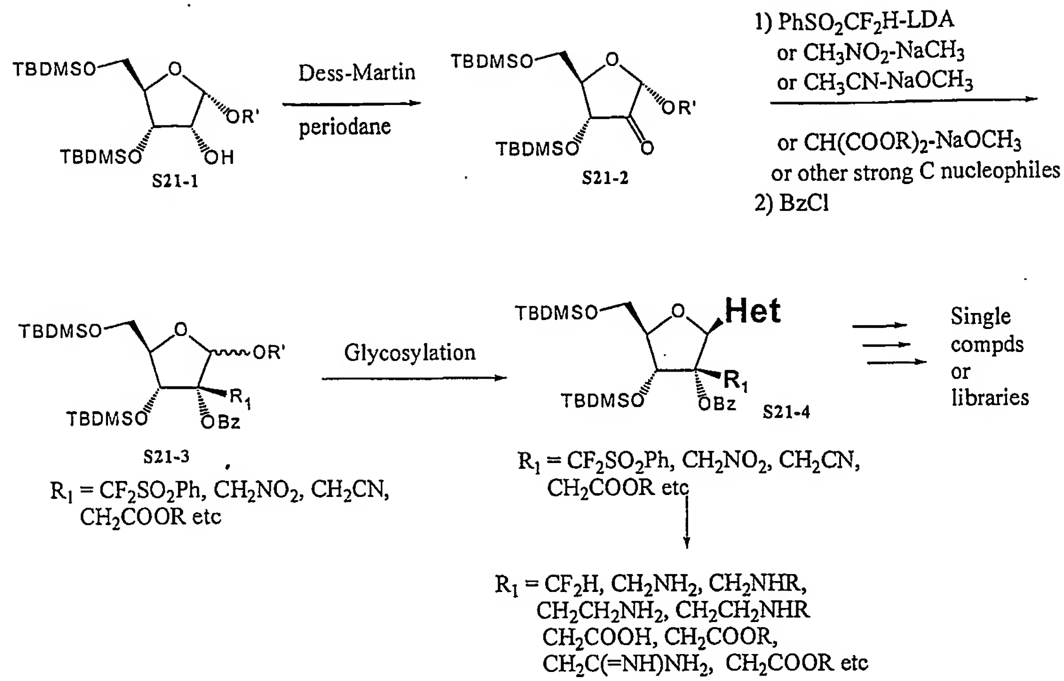
In another example, it is contemplated that sugar modifications may be employed to provide improved biochemical properties to contemplated compounds. For example, where the compound is employed as an inhibitor of an RNA-dependent RNA polymerase (*e.g.*, NS5B of HCV), a 2'-beta (and especially a 2'-beta methyl or 2'-beta hydroxymethyl) modification may be included to improve antiviral activity. While not wishing to be bound to a particular theory, it is contemplated that such modification improves the selectivity of contemplated compounds to the HCV polymerase (over other polymerases) as well as decreases the Km of the compound (as compared to the same compound without the modification).

In still further contemplated modifications at the sugar, it should be recognized that all modifications may be employed to increase one or more pharmacological (*e.g.*, half-life time, absorption, bioconversion, etc.) or biochemical parameters (*e.g.*, solubility, electrical charge, selectivity to a structure interacting with contemplated compounds, etc.) of contemplated compounds, and all known modifications are contemplated suitable for use herein. Similarly, it should be recognized that the heterocyclic base may also be modified to increase one or more pharmacological or biochemical parameters of contemplated compounds. For example suitable modifications on OH groups may include esterifications, and modifications on NH2 groups may include amidations.

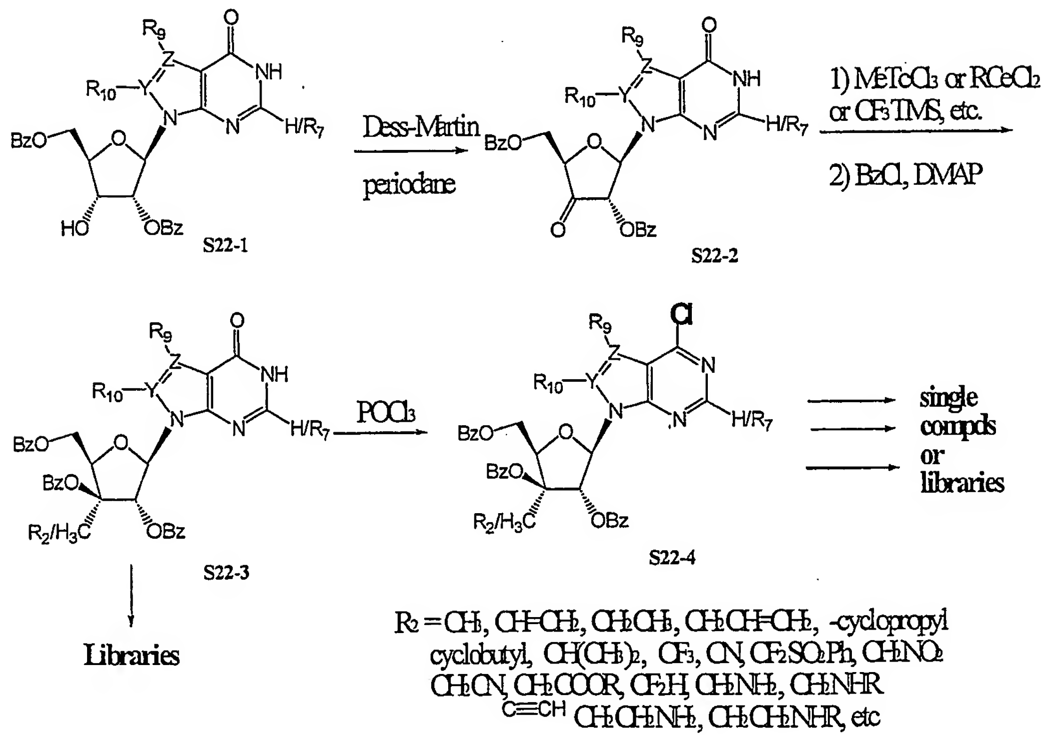
Modification Of The Sugar To Yield C_2'- And/Or C_3'-Substituted Sugars

It is generally contemplated that all known procedures and synthetic schemes for modification of a sugar to yield a C_2'- and/or C_3'-substituted sugar are suitable for use herein, and exemplary protocols may be found in "Modern Methods in Carbohydrate Synthesis" by Shaheer H. Khan (Gordon & Breach Science Pub; ISBN: 3718659212), in U.S. Pat Nos. 4,880,782 and 3,817,982, in WO88/00050, or in EP199,451.

It should further be appreciated that the modification on the sugar portion of a nucleoside may be introduced when the sugar is covalently coupled to the heterocyclic base, or before coupling of the sugar to the heterocyclic base. Exemplary methods of introducing a substituents into the C_2'- or C_3'-position is depicted in **Schemes 9** and **10** below.

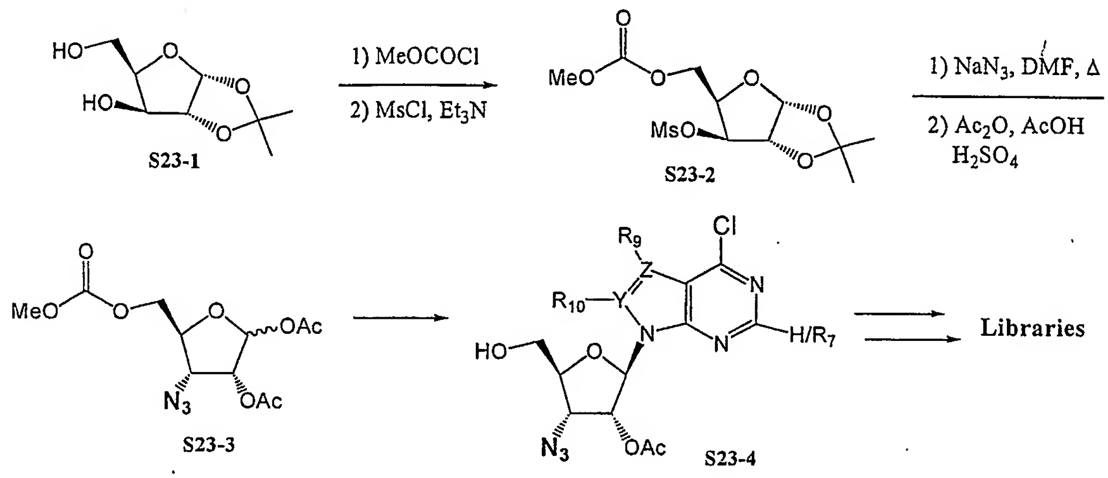


Scheme 9



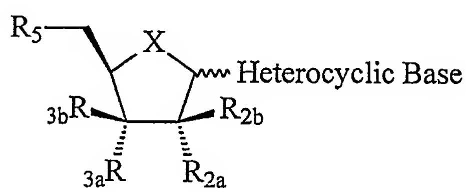
Scheme 10

Similarly, where azido sugars are desired, the azido group may be introduced via the corresponding azido salt in a reaction with a suitable protected sugar as shown in **Scheme 11** below.



Scheme 11

Thus, especially preferred alternative sugars for contemplated nucleosides include those having the general **Formula 5**.



Formula 5

wherein Heterocyclic Base is any one of the heterocyclic bases contemplated herein; X is O, S, or CH_2, R_{2a} and R_{3a} are independently H, OH, SH, NH_2, NHR, OR, SR, CH_2OH, N_3, NH_2, COOH, halogen, or $P(O)(OR)_2$ with R being alkyl, acyl, or alkenyl (each of which may optionally be substituted); R_{2b} and R_{3b} are independently H, OH, CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH_2(CH_2)_{2\text{-}5}CH_3$, C_1-C_8 alkyl, alkenyl, or alkynyl (which may be linear, branched, or cyclic), C_5-C_{12}-aromatic or heterocyclic system, halogen (*i.e.*, F, Cl, Br, I), CF_3, CHF_2, CCl_3, $CHCl_2$, CH_2Cl, CH_2OH, CN, CH_2CN, CH_2NH_2, CH_2NHR, CH_2OR, CHO, CH_2COR, N_3, or NH_2, SH, NH_2, NHR, OR, SR, CH_2OH, N_3, NH_2, COOH, halogen, or $P(O)(OR)_2$ with

R being alkyl, acyl, or alkenyl (each of which may optionally be substituted), and wherein R_5 is OH, monophosphate, diphosphate, triphosphate, or analogs thereof (*e.g.*, phosphonate, boranophosphate, or thiophosphate).

Contemplated Prodrugs and Metabolites

It should still further be appreciated that the compounds according to the inventive subject matter also include prodrug forms, phosphorylated forms (most preferably at the C5'-atom) and/or metabolites. Particularly suitable prodrug forms of contemplated compounds may include a moiety that is covalently coupled to at least one of the C2'-atom, C3'-atom, and C5'-atom, thereby replacing the OH group at the at least one of the C2'-atom, C3'-atom, and C5'-atom, wherein the moiety is preferentially cleaved from the compound in a target cell (*e.g.*, Hepatocyte) or a target organ (*e.g.*, liver). While not limiting to the inventive subject matter, it is preferred that cleavage of the prodrug into the active form of the drug is mediated (at least in part) by a cellular enzyme, particularly receptor, transporter, and cytochrome-associated enzyme systems (*e.g.*, CYP-system).

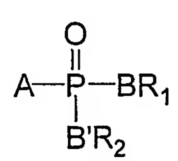
Especially contemplated prodrugs comprise a cyclic phosphate, cyclic phosphonate and/or a cyclic phosphoamidate, which are preferentially cleaved in a hepatocyte to produce the corresponding nucleotides. There are numerous such prodrugs known in the art, and all of those are considered suitable for use herein. However, especially contemplated prodrug forms are disclosed in WO 01/47935 (Novel Bisamidate Phosphonate Prodrugs), WO 01/18013 (Prodrugs For Liver Specific Drug Delivery), WO 00/52015 (Novel Phosphorus-Containing Prodrugs), and WO 99/45016 (Novel Prodrugs For Phosphorus-Containing Compounds), all of which are incorporated by reference herein. Consequently, especially suitable prodrug forms include those targeting a hepatocyte or the liver.

Still further particularly preferred prodrugs include those described by Renze et al. in Nucleosides Nucleotides Nucleic Acids 2001 Apr-Jul;20(4-7):931-4, by Balzarini et al. in Mol Pharmacol 2000 Nov;58(5):928-35, or in U.S. Pat. No. 6,312,662 to Erion et al., U.S. Pat. No. 6,271,212 to Chu et al., U.S. Pat. No. 6,207,648 to Chen et al., U.S. Pat. No. 6,166,089 and U.S. Pat. No. 6,077,837 to Kozak, U.S. Pat. No. 5,728,684 to Chen, and published U.S. Application with the number 20020052345 to Erion, all of which are incorporated by reference herein. Alternative contemplated prodrugs include those comprising a phosphate and/or phosphonate non-cyclic ester (SATE ester, pivaloyl ester,

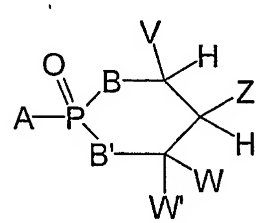
etc.), and an exemplary collection of suitable prodrugs is described in U.S. Pat. No. 6,339,154 to Shepard et al., U.S. Pat. No. 6,352,991 to Zemlicka et al., and U.S. Pat. No. 6,348,587 to Schinazi et al. Still further particularly contemplated prodrug forms are described in FASEB J. 2000 Sep;14(12):1784-92, Pharm. Res. 1999, Aug 16:8 1179-1185, and Antimicrob Agents Chemother 2000, Mar 44:3 477-483, all of which are incorporated by reference herein.

Thus, particularly preferred prodrug forms will comprise a moiety covalently coupled to at least one of the C2'-atom, C3'-atom, and C5'-atom, wherein at least part of the moiety is preferentially cleaved from the compound in a target cell or target organ. As used herein, the term “preferentially cleaved…in a target cell or target organ” means that cleavage occurs in a particular target cell or target organ at a rate that is at least 3 times, more typically at least 10 times, and most typically at least 50 times higher than in a non-target cell or non-target organ. The term “target cell” or “target organ” as used herein refers to a cell or organ that is infected with a virus, and especially includes a hepatocyte infected with an HCV virus. Cleavage may be mediated by enzymes (but also by non-enzymatic processes, *e.g.*, via reductive cleavage), and it is particularly preferred that enzymatic cleavage is mediated by a liver-specific enzyme system (*e.g.*, CYP system). Consequently, it should be appreciated that certain prodrug forms of contemplated compounds may be cleaved in a target cell and/or target organ to provide a nucleotide analog. Alternatively, prodrugs may also be converted to the corresponding nucleoside (*e.g.*, where the moiety does not include a phosphorus atom).

An exemplary preferred prodrug of contemplated compounds may therefore include a moiety according to **Formula M1** or **M2** (covalently coupled to the compound, typically to the C5’-atom, C2’-atom, and/or C3’-atom)



M1



M2

wherein A in M1 or M2 is O or CH_2 and replaces the 5’-OH group of the compound of Formulae 1 -5; B and B’ are independently O or NH, and where B is NH then R_1 or R_2 is an amino acid that forms a peptide bond with the N atom of the NH; and R_1, R_2, V, W, and

W' are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, alkaryl, each of which is optionally substituted, and Z is hydrogen, CHWOH, CHWOCOW', SW, or CH_2aryl. Especially preferred compounds according to Formula M2 are those in which A is O or CH_2, B and B' are independently O or NH, and in which Z, W, and W' are H and V is m-Chlorophenyl.

With respect to metabolites of contemplated compounds, it should be recognized that all metabolites that have a desirable therapeutic effect, and especially an antiviral effect are deemed suitable. Consequently, particularly suitable metabolites will generally include 5'-phosphates (*e.g.*, monophosphate, diphosphate, and/or triphosphate esters), which may or may not be generated by an enzyme (*e.g.*, kinase, oxidase). Further metabolites include those that are generated via enzymatic action on the heterocyclic base (*e.g.*, via deaminase, deamidase, or hydroxylase).

Use of Contemplated Compounds

It is generally contemplated that all libraries will comprise one or more nucleosides that have numerous biological activities, and especially contemplated biological activities include *in vitro* and *in vivo* inhibition of DNA and/or RNA polymerases, reverse transcriptases, and ligases. Therefore, contemplated nucleosides will exhibit particular usefulness as *in vitro* and/or *in vivo* antiviral agents, antineoplastic agents, and immunomodulatory agents.

Particularly contemplated antiviral activities include at least partial reduction of viral titers of respiratory syncytial virus (RSV), hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex type 1 and 2, herpes genitalis, herpes keratitis, herpes encephalitis, herpes zoster, human immunodeficiency virus (HIV), influenza A virus, Hanta virus (hemorrhagic fever), human papilloma virus (HPV), yellow fever virus, and measles virus. The anti-HCV activity of the nucleosides and libraries were tested by Replicon and BVDV cell-line based assays. The HCV NS5B polymerase activity were tested for the mono-, di- and triphosphates of the nucleosides or 5'-methylenephospnonate derivatives. The compounds and libraries were tested for their replication of Hepatitis C virus RNA by cell-line based HCV Replicon assay as described in V. Lohmann, F. Korner, J.-O. Koch, U. Herian, L. Theilmann, R. Bartenschlager, "Replication of a Subgenomic Hepatitis C virus RNAs in a Hepatoma Cell Line", Sciences, 1999, *285*, 110. Especially contemplated immunomodulatory activity

includes at least partial reduction of clinical symptoms and signs in arthritis, psoriasis, inflammatory bowel disease, juvenile diabetes, lupus, multiple sclerosis, gout and gouty arthritis, rheumatoid arthritis, rejection of transplantation, giant cell arteritis, allergy and asthma, but also modulation of some portion of a mammal's immune system, and especially modulation of cytokine profiles of Type 1 and Type 2. Where modulation of Type 1 and Type 2 cytokines occurs, it is contemplated that the modulation may include suppression of both Type 1 and Type 2, suppression of Type 1 and stimulation of Type 2, or suppression of Type 2 and stimulation of Type 1.

Where contemplated nucleosides are administered in a pharmacological composition, it is contemplated that suitable nucleosides can be formulated in admixture with a pharmaceutically acceptable carrier. For example, contemplated nucleosides can be administered orally as pharmacologically acceptable salts, or intravenously in a physiological saline solution (*e.g.*, buffered to a pH of about 7.2 to 7.5). Conventional buffers such as phosphates, bicarbonates or citrates can be used for this purpose. Of course, one of ordinary skill in the art may modify the formulations within the teachings of the specification to provide numerous formulations for a particular route of administration. In particular, contemplated nucleosides may be modified to render them more soluble in water or other vehicle, which for example, may be easily accomplished with minor modifications (salt formulation, esterification, *etc.*) that are well within the ordinary skill in the art. It is also well within the ordinary skill of the art to modify the route of administration and dosage regimen of a particular compound in order to manage the pharmacokinetics of the present compounds for maximum beneficial effect in a patient.

In certain pharmaceutical dosage forms, prodrug forms of contemplated nucleosides may be formed for various purposes, including reduction of toxicity, increasing the organ or target cell specificity, etc. Among various prodrug forms, acylated (acetylated or other) derivatives, pyridine esters and various salt forms of the present compounds are preferred. One of ordinary skill in the art will recognize how to readily modify the present compounds to pro-drug forms to facilitate delivery of active compounds to a target site within the host organism or patient. One of ordinary skill in the art will also take advantage of favorable pharmacokinetic parameters of the pro-drug forms, where applicable, in delivering the present compounds to a targeted site within the host organism or patient to maximize the intended effect of the compound.

In addition, contemplated compounds may be administered alone or in combination with other agents for the treatment of various diseases or conditions. Combination therapies according to the present invention comprise the administration of at least one compound of the present invention or a functional derivative thereof and at least one other pharmaceutically active ingredient. The active ingredient(s) and pharmaceutically active agents may be administered separately or together and when administered separately this may occur simultaneously or separately in any order. The amounts of the active ingredient(s) and pharmaceutically active agent(s) and the relative timings of administration will be selected in order to achieve the desired combined therapeutic effect.

Experiments and Data

SYNTHESIS OF EXEMPLARY COMPOUNDS

Scheme 1

Synthesis of the Dess-Martin Reagent

1,1,1-Triacetoxy-1,1-Dihydro-1,2-Benziodoxol-3-(1H)-one: A 3L 3 neck RB flask was fitted with a mechanical stirrer, a heating mantle, a thermometer with adapter and a sodium hydroxide scrubber system. The vessel was charged with 100 g (0.403 mol) of 2-Iodobenzoic acid and 860 ml of 0.73 Molar sulfuric acid solution. The resultant white suspension was stirred and the pot temperature was increased to 55 °C at which point the vessel was charged with 87.3 g (0.523 mol) of potassium bromate, added in small portions over a 40 min time period. After the addition was completed the pot temperature of the thick orangish-amber suspension was increased to 70 °C, and the condition was maintained for a 3.5h time period. The reaction mixture was allowed to cool to ambient conditions. The reaction mixture was cooled to –1°C and maintained for a 0.5 h. The filter cake was washed with 1L of water, followed by 2 x 100 ml of ethyl alcohol, and then finally with 2 x 150 ml of diethyl ether. The cake was dried at high vacuum (~0.1mmHg)/ambient temperature for 24 h. 101.56 g of white powder was obtained in 90% yield.

A 1L 3 neck RB flask was fitted with a mechanical stirrer, a heating mantle, a thermometer with adapter, and a nitrogen inlet/outlet. The vessel was charged with 400 ml (4.24 mol) of acetic anhydride (4ml/g) and 0.5 g (0.00263 mol) of p-toluenesulfonic acid-monohydrate under nitrogen atmosphere and stirring. The reaction mixture was charged with

100 g (0.357 mol) of 1-hydroxy-1,2-benziodoxol-3-(1H)-one. The resulting pale yellow suspension (pot temp = 23 °C) was then heated to 80°C for 2h. The reaction mixture was allowed to cool to ambient temperature and then cooled to –2 °C. The white suspension was stirred at –2 °C for 0.5 h and filtered. The filter cake was washed with 5 x 50 ml of diethyl ether and then quickly transferred to an amber bottle under an argon atmosphere. The bottle was subsequently stored under refrigeration at < 5°C. 136.27 g of the desired product was obtained as a white solid in 90% yield. [This Dess-Martin reagent was synthesized in a large scale by a well modified and practical protocol based on the reported procedure: Cook, G. P.; Greenberg, M. M. J. Org. Chem. 1994, 59, 4704-4706].

Synthesis of 1',3',5'-Tri-*O*-benzoyl-D-ribofuranose was synthesized by a modified procedure based on the literature [Brodfuehrer, P. R.; Sapino, C., Jr.; Howell, H. G. J. Org. Chem. 1985, 50, 2598].

Synthesis of 1',3',5'-Tri-O-benzoyl-D-2-Ketoribofuranose: A 3L 3-necked RB flask was fitted with a mechanical stirrer, a thermometer with adapter, and a nitrogen inlet/outlet, to which was added 201 g (0.474 mol) of 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3-(1H)-one (Dess-Martin reagent) and 1000 mL (15.60 mol) of dichloromethane under nitrogen atmosphere. The reaction mixture was cooled to –1 °C, and 100 g (0.216 mol) of 1',3',5'-tri-O-benzoyl-D-ribofuranose was added. The resultant reaction mixture was stirred at room temperature for 24 hours and concentrated. The resultant residue was triturated with diethyl ether. The resultant ether triturate was filtered through a pad of Celite, and then treated with 1L of 1.0 M sodium thiosulfate solution. The organic phase was washed with sodium thiosulfate solution, and saturated sodium bicarbonate solution followed by brine. The organic phase was dried over magnesium sulfate and concentrated. The clear viscous pale yellow oily residue was subsequently dissolved in 2 L of dichloromethane. The solution was further treated with 500g of magnesium sulfate for 24 h and concentrated. The residue was further dried under high vacuum to provide 95.47 g (96%) of the desired product as a white foam. [Cook, G. P.; Greenberg, M. M. J. Org. Chem. 1994, 59, 4704-4706].

Synthesis of 1',2',3',5'-Tri-*O*-benzoyl-2-beta-*C*-methyl-D-ribofuranose : A 5L 3 neck RB flask was fitted with a mechanical stirrer, a thermometer with adapter, an additional funnel, a nitrogen inlet/outlet and a cooling bath. The vessel was charged with 2800 ml (26.74 mol) of diethyl ether (17 ml/g based on TiCl4) to which a continuous gentle stream of

nitrogen was passed over. The reaction mixture was stirred and cooled to –78 °C. 164.2g (0.868 mol) of titanium (IV) chloride was added drop wise over 1h. The resulting clear light yellow reaction mixture was treated with 289 ml (0.868 mol) of 3 Molar methyl magnesium bromide in diethyl ether drop wise. The reaction mixture was allowed to slowly warm to a pot temperature of –30 °C at which point 100 g (0.217 mol) of 1,3,5-tri-*O*-benzoyl-alpha-D-2-keto-ribofuranose in 200 ml of diethyl ether (2ml/g) was added drop wise. The reaction mixture was allowed to stir at –30 °C for a 4 h. The organic phase was separated, and the aqueous phase was extracted with 3 x 2000 ml of diethyl ether. The combined organic phase was washed with water and then dried over magnesium sulfate. The organic solution was concentrated, and the residue was subsequently dried at high vacuum (~0.1mmHg)/ambient temperature for a 24 h to provide 100.3 g (97%) of intermediate compounds as a clear viscous oil, which were then used directly for the next step.

A 2L 3 neck RB flask was fitted with a mechanical stirrer, a thermometer with adapter, an addition funnel, and a nitrogen inlet/outlet to which were added 6.63 g (0.0543 mol) of 4-dimethlyaminopyridine and 500 ml (7.80 mol) of dichloromethane under nitrogen atmosphere. 85 ml (0.613 mol) of triethylamine was added followed by the addition of 12.6 ml (0.1086 mol) of benzoyl chloride drop wise. 25.87 g (0.0543 mol) of sugar intermediates 5 and 6 obtained above in 125ml of dichloromethane (5ml/g) was added drop wise. The resulting clear light amber reaction mixture was allowed to stir at ambient conditions for 3h to complete the reaction (TLC analysis on silica gel, 4:1 Hex/EtOAc). The reaction mixture was diluted with 2.5L of diethyl ether, and the clear pale amber solution was partitioned with a 750ml portion of 1 Molar HCl solution in an extraction vessel. The organic phase was separated and washed with 2 x 500 ml of 1 Molar HCl solution, followed by a 500 ml of water and 2 x 500ml portions of saturated sodium bicarbonate solution. The organic solution was dried over sodium sulfate and concentrated. The remaining residue was subsequently pumped at high vacuum/ambient temperature for a 14 h. The crude product was flashed through a 740 g plug of silica gel (20:1) packed and loaded with 9:1 hexane/ethyl acetate and eluted with a gradient from 9:1 to 4:1 hexane/ethyl acetate. The desired fractions were combined and evaporated on a rotary evaporator at 26mmHg/bath temperature 35°C and the remaining residue was pumped at high vacuum (~0.1mmHg)/ambient temperature for a 14 h to provide 17.03 g (54%) of the desired product 7 as a pale yellow solid. 1H NMR (CDCl3) d 1.97 (s, 3H), 4.56 (dd, 1H, J = 4.8, 12.0 Hz), 4.68 (dd, 1H, J = 4.8, 12.0 Hz), 4.80 (m, 1H),

5.98 (d, 1H, J = 8.0 Hz), 7.02 – 8.15 (m, 21 H). [Wolfe, M. S.; Harry-O'kuru, R. E. Tetrahedron Lett. 1995, 36, 7611-7614; Harry-'Okuru, R. E.; Smith, J. M.; Wolfe, M. S. J. Org. Chem. 1997, 62, 1754-1759].

6-Chloro-9*H*-(2'-β-*C*-methyl-2',3',5'-tri-*O*-benzoyl-*β*-D-ribofuranosyl)purine (12-1) was prepared based on the literature procedure with modification (P. Franchetti, L. Cappellacci, S. Marchetti, L. Trincavelli, C. Martini, M. R. Mazzoni, A. Lucacchini, M. Grifantrini, *J. Med. Chem.* **1998**, *41*, 1708-1715). To a stirred mixture of 6-chloropurine (1.82 g, 11.08 mmol) and 2'-β-C-methyl-1,2,3,5-tetra-*O*-benzoyl-D-ribose (6.44 g, 35.46 mmol) in anhydrous CH_3CN (200 ml) under an Argon atmosphere was added 1,8-diazabicyclo[5.4.0]undec-7-ene (5.39 g, 35.46 mmol) at room temperature. The stirred mixture was cooled to 0 °C under Argon atmosphere. Me_3SiOTf (10.49 g, 47.23 mmol) was added to the reaction mixture slowly during a 15 minute period at 0 °C. The reaction mixture was then warmed to room temperature during a 30 minute period. The resulting reaction mixture was heated at 60 °C for 4 h and concentrated to dryness. The residue was partitioned between ethyl acetate and saturated $NaHCO_3$ (300/200 ml). The organic phase was separated, and the aqueous phase was extracted in ethyl acetate. The combined organic phase was washed with brine, dried over anhydrous sodium sulfate, and concentrated to dryness. The resultant residue was purified by flash chromatography on a silica gel column using hexane → EtOAc as the eluent. The pure fractions were collected and concentrated to dryness to provide 6.60 g (95%) of the titled compound. ^1H NMR ($CDCl_3$) δ 1.61 (s, 3 H), 4.77 (m, 1 H), 4.93 (m, 2 H), 6.21 (d, 1 H), 6.82 (s, 1 H), 7.30–7.62 (m, 9 H), 7.94–8.12 (m, 6 H), 8.30 (s, 1 H), 8.79 (s, 1 H).

Modification of the compound 12-1 to yield compounds **(13-1)** was performed under conditions substantially similar to those described in J.W. Labadie, D Tueting, and J.K. Stille. *J. Org. Chem.* 48,4634 (1983), or in A.M. Echavarren and J.K. Stille. *J. Am. Chem. Soc.* 110, 1557 (1988)) for Stille reactions, or those described in R.F. Heck. *Org. React.* N.Y. 27, 345 (1982), or J.E. Plevyak and R.F. Heck. *J. Org. Chem.* 43, 2454 (1978) for Heck reaction, or those described in N. Miyaura, T. Yanagi, and A. Suzuki. *Synth. Commun.* 11, 513 (1981); A. Suzuki. *Pure Appl. Chem.* 57, 1749 (1985), or M. Sato, N. Miyaura, and A. Suzuki. *Chem. Lett.* 1405 (1989) for Suzuki reaction. Similarly, where a Grignard reaction was employed to yield compounds 13-1, protocols substantially similar to those described in Grignard V.

*Compt. Rend., **1900**, 130*, 1322, or Shirley, D.A. *Org. React.*, 1954, 8, 28-58 were used. Subsequent deprotection using methanolic ammonia was performed using a general deprotection protocol well known to a person of ordinary skill in the art to provide library and library compounds (**13-3**).

Scheme 2

Toluene-4-sulfonyl chloride (3.8 g, 20 mmol) was added to a suspension of **S16-1** (Y-R_{10} is C-H, and Z-R_9 is N-null) (10 mmol), triethylamine (2.87 ml, 20 mmol), and DMAP (244 mg, 2 mmol) in 150 ml of anhydrous dichloromethane at 0 °C under argon. The reaction mixture was stirred at room temperature overnight. A clear brown solution was obtained. The reaction mixture was diluted with dichloromethane and washed successively with water and brine. The organic layer was dried over anhydrous sodium sulfate, concentrated to a small volume, and then added to 1,000 ml of hexanes with vigorous stirring at room temperature. The resulting precipitate was filtered and washed with hexanes to give product **S16-2** as a white solid.

A solution of **S16-2** (1 mmol), lithium chloride (85 mg, 2 mmol), and of $Pd(PPh_3)_4$ (231 mg, 0.2 mmol) in 15 ml of anhydrous dioxane was stirred under argon at room temperature for 10 min. Tributyl(vinyl)tin (1.46 ml, 5 mmol) was added and the mixture was heated under reflux for 4 hours. The solvent was evaporated in vacuo, and the residue was purified by flash chromatography on a silica gel column using chloroform-methanol (50:1) as an eluent to give pure product **S16-3** as yellow foam.

To a solution of **S16-3** (1 equiv) in chloroform-methanol (50:1) or ethanol was added amino acid ester, amines, thiophenols, mercaptans and other nucleophiles (1 equiv) at room temperature or elevated temperature. The resulting mixture was stirred at room temperature for 1 hour. The solvent was removed in vacuo, and the residue was purified by flash chromatography on a silica gel column using chloroform-methanol (50:1) as an eluent to give the corresponding 6-substituted ethyl purine nucleoside derivatives **S16-4**.

Schemes 3 and 4

Compounds and libraries **S14-4** And **S15-3** were prepared following substantially similar protocols as described for Schemes 1 and 2 above.

Scheme 5

5,7-Dihydroxy-1(2)-methylpyrazolo[4,3-d]pyrimidine **S17-1** was synthesized by heating 4-amino-1(2)-methylpyrazole-3-carboxyamide (11.61 g, 82.85 mmole) and urea (12 g) at 120° C for 20 minutes. After cooling, the crude product was dissolved in 2N NaOH (200 ml) and acidified with AcOH to pH 3-4. The precipitated solid was filtered, washed with water and dried at high vacuo over solid NaOH to give 11.01 g (80%) of light brown solid.

4-(2,3,5-Tri-*O*-benzoyl-*β*-D-ribofuranosyl)-1-methylpyrazolo[4,3-d]pyrimidine-5,7(6*H*)-dione S17-2 and 4-(2,3,5-Tri-*O*-benzoyl-*β*-D-ribofuranosyl)-2-methylpyrazolo[4,3-d]pyrimidine-5,7(6*H*)-dione **S17-3**. A mixture of **S17-1** (11.01 g, 66.28 mmole), ammonium sulfate (0.2 g), dry pyridine (50 ml) and 1,1,1,3,3,3-hexamethyldisilazane (HMDS, 200 ml) was heated at reflux for 18 h. The reaction mixture was cooled to room temperature and evaporated to dryness. The residue was co-evaporated with dry xylene (100 ml) and mixed with dry acetonitrile (200 ml). Ribose derivatives **S1-2** (70.00 mmole) were added and cooled to 0° C under argon atmosphere. To this cold stirred mixture was added trimethylsilyl trifluoromethanesulfonate (TMS triflate, 15.00 ml) dropwise during 30 minutes period. After the addition, the solution was gradually warmed to room temperature and stirred overnight. Methanol (30 ml) was added, stirred for 15 minutes and evaporated to dryness. The residue was dissolved in ethyl acetate (500 ml), and washed successively with saturated $NaHCO_3$ solution (3x200 ml), water (300 ml) and brine (200 ml). The organic extract was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography over silica gel using $CHCl_3 \rightarrow$ EtOAc as the eluent. Two products were isolated. The fast moving product (33%) was assigned as compound **S17-2** (1-methyl). The slow moving product (50%) was assigned as compound **S17-3** by 2D NMR.

A mixture of compound **S17-2** (30.16 mmole) and Lawesson's reagent (24.40 g, 60.33 mmole) in dry pyridine (150 ml) was heated at reflux for 12 h. The reaction mixture was cooled to room temperature and evaporated to dryness. The residue was treated with sat. $NaHCO_3$ solution (300 ml) and extracted with EtOAc (300 ml). The organic extract was washed with brine (150 ml), dried over anhydrous sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography over silica gel using $CHCl_3 \rightarrow$ EtOAc as the eluent to give 15.48 g (82%) of S17-4 as yellow foam. **S17-5** was synthesized by the same approach.

Compound **S17-4** (23.96 mmole) was allowed to stir with methanolic ammonia (250 ml) in a steel bomb at room temperature for 2 days. The steel vessel was cooled, opened and the solution was evaporated to dryness. The residue was crystallized from methanol/ethyl acetate to give 6.02 g (80%) of light brownish solid. A solution of the resultant compound (17.52 mmole) in dry DMF (100 ml) was treated with N,N-diisopropylethylamine (2.46 g, 19.00 mmole) at room temperature. After 30 minutes, methyl iodide (5 ml) was added and the stirring continued for 12 h. The reaction mixture was filtered, and the solid was washed with dry DMF (50 ml). The combined filtrate was evaporated to dryness. The residue was purified by flash chromatography over silica gel using $CHCl_3 \rightarrow$ MeOH as the eluent to give 4.71 g (82%) of yellow solid. A suspension of this compound (13.72 mmole) and psMMTCl-resin (5.56 g) in dry pyridine (40 ml) was shacked at room temperature for 3 days. The reaction mixture was quenched with methanol (20 ml) and shacked for an additional 30 minutes. The resin was filtered, washed with dry DMF (2x20 ml), MeOH (2x20 ml), and CH_2Cl_2 (2x20 ml). The resin was dried in vacuo at 45° C overnight to give resin **S17-6**. Resin **S17-7** was synthesized by the same approach.

A suspension of resin **S17-6** or **S17-7** (100 mg) and various amines and other nucleophilic building blocks (see building block lists) (2 equiv.) were heated at 60° C for 12 and filtered. The resins were washed with CH_2Cl_2 (2x10 ml), MeOH (2x10 ml), DMF (2x10 ml) and CH_2Cl_2 (2x10 ml). These resins were shaken with 5% TFA (2 ml) at room temperature for 1 h and filtered. The filtrate was evaporated to dryness. The residues were co-evaporated with dry toluene (2x5 ml) to give 15-25 mg of libraries **S17-8** or **S17-9** as amorphous solid single compounds.

Scheme 6

Compound **S18-1** was prepared by the similar procedure as reported (P. Franchetti, L. Cappellacci, S. Marchetti, L. Trincavelli, C. Martini, M. R. Mazzoni, A. Lucacchini, M. Grifantrini, J. Med. Chem. 1998, 41, 1708-1715). A solution of S18-1 (1 equiv, DMAP (100 mg), dichloromethane (30 ml) and triethylamine (6.8 ml) was stirred at room temperature for 30 minutes. 2,4,6-tris(isopropyl)benzenesulfonyl chloride (TIP-Cl, 4.24 g, 2 equiv) were added. The resultant mixture was stirred at room temperature for 24 hours. 2 ml of methanol were added to consume the excess amount of TIP-Cl, shaken and filtered. The solution was concentrated and purified by chromatography on a silica gel column providing compound

S18-2 as white foam. This compound was reacted in parallel with various amines and other nucleophiles (see building block lists) at room temperature or elevated temperature. The resulted compounds were treated with ammonia to provide the final single compounds **S18-3**. Compounds **S19-3** are synthesized by the similar approach.

Scheme 7

Scheme 7 followed the protocol of Scheme 6 with the exception that the positions of the radicals Z and R_1 in the heterocyclic base were switched (relative to the heterocyclic base shown in Scheme 6). Otherwise, the same conditions and procedures were employed.

Scheme 8

To a solution of ribofuranose-1-acetate derivatives **S1-2** (9.91 mmol) in 50 ml of dimethylformamide was added 0.97 g of sodium azide (14.87 mmol). The mixture was stirred at 115 °C for 16 hours under argon and then evaporated. The residue was extracted by chloroform and dried over sodium sulfate. The resultant compound was treated with NaCN to give compound **S20-1**.

To a suspension of polystyrene monomethoxytrityl chloride resin (1 g, 1.73 mmol/g) in pyridine (4 ml), a solution of β-D-ribofuranosyl-1-azide derivatives **S20-1** (3.46 mmol) in pyridine (4 ml) was added which was followed by the addition of 4-dimethylaminopyridine (0.122 g, 1 mmol). The reaction mixture was shaken well for 48 h at room temperature. The resin was filtered and washed with CH_2Cl_2 (3x25 ml) and a mixture (8.5:1:0.5) of MeOH, CH_2Cl_2 and diisopropylethylamine. The product resin was then dried over KOH under vacuum for 16 h. The loading efficiency was 85% (1.46 mmol alcohol loaded). To a suspension of this resin in DMF were added excess amounts of TBDMS-Cl (1.29 g, 8.65 mmol) and imidazole (1.17 g, 17.3 mmol). The reaction mixture was shaken for 16 h at room temperature. The resin was filtered and washed with DMF (3x10 ml), MeOH (3x10 ml) and CH_2Cl_2 (3x10 ml). The resin was then dried over KOH under vacuum for 16 h. To a suspension of the resultant resin (1.1 g) in a mixture (7.4 ml) of THF and water (9:1), a solution (1 M) of PMe_3 in THF (2.6 ml) was added and shaken well at room temperature for 6 h. The resin was filtered and then washed with THF and water mixture (1:1, 3x10 ml), MeOH (3x10 ml), CH_2Cl_2 (3x10 ml). The resin **S20-2** was then dried over KOH under vacuum for 16 h.

The resin **S20-2** (1 g) was suspended in a solution of diisopropylethylamine in CH_2Cl_2 (5 ml, 20%v/v) and cooled to 0-5 °C (ice-cold water). It was then treated with a CH_2Cl_2 solution (5 ml, 1 M) of cyanuric chloride. The resin suspension was shaken at room temperature for 1 h and filtered using a sintered funnel. The resin S20-3 was washed with CH_2Cl_2 (3x25 ml) and dried over KOH under vacuum for 16 h.

To a suspension of resins **S20-3** (0.05 g) in an NMP solution of DIPEA (0.75 ml, 20%v/v), an NMP solution (0.75 ml, 1M) of amines (see building block lists) was added and the reaction mixture was shaken well at room temperature for 2 h. The resin was then washed with NMP (3x10 ml), MeOH (3x10 ml) and CH_2Cl_2 (3x10 ml). The resins S20-4 were then dried over KOH under vacuum for 16 h. To a suspension of resins S20-4 (0.05 g) in an NMP solution of DIPEA (0.75 ml, 20%v/v) was added an NMP solution (0.75 ml, 1M) of amines (see building block lists) and the reaction mixture was shaken well at 80 °C for 6 h. The resins were then washed with NMP (3x10 ml) and CH_2Cl_2 (3x10 ml) and then dried over KOH under vacuum for 16 h. A suspension of above resins (0.05 g) in THF solution (1.5 ml, 1 M) of TBAF was shaken well at room temperature for 16 h. The resins were filtered and treated with DMF-acetic acid mixture (1.5 ml, 9:1) for 60 seconds and filtered. The resins were washed with DMF-water mixture (9:1, 3x10 ml), MeOH (3x10 ml) and CH_2Cl_2 (3x20 ml) and dried over KOH under vacuum for 16 h. A suspension of above resins (0.05 g) in CH_2Cl_2 solution (1.5 ml, 1.5%) of TFA were shaken well at room temperature for 60 seconds and filtered. The resins were further washed with MeOH (2x1 ml) and the combined filtrates were evaporated to dryness to give libraries **S20-5**. These libraries were synthesized by the parallel 96-well synthesizer. Twelve plates (96 X 12) were synthesized having 95-98% over 60% purity detected by LC-MS spectrometry.

Exemplary Amino Building blocks (R-NH_2 or RNHR) used for the libraries

1-(Benzyl)benzylamine, 2-phenyl-n-propylamine, m-trifluorobenzylamine, 2,2-diphenylethylamine, cyclobutylamine, methylcyclohexylamine, 2-methylpropylamine, allylcyclopentanylamine, N-methyl-4-piperidinylmethylamine, 4-hydroxypiperidine, 3-hydroxypiperidine, 1-benzylpiperazine, p-methoxybenzylamine, N,N-

bis(isopropyl)aminoethylamine, 2-ethylhexylamine, 5-methyl-2-furanosylmethylamine, N,N-dimethylaminopropylamine, 3-(N,N-dimethylamino)-2,2-dimethylpropylamine, 2-methylbutylamine, o-ethoxybenzylamine, 3-(2-methyl-N-piperidinylpropylamine, 1-(2-aminoethyl)pyrrolidine, 2-morpholinylethylamine, N4-hydroxyethylpiperazine, N-methylethylenediamine, 3-morpholinylpropylamine, pyridinyl-2-ethylamine, butylamine, hexylamine, methylamine, 2-hydroxyethylamine, N,N-dimethylethylenediamine, 3-methoxypropylamine, 2-methoxylethylamine, ethylamine, 2-isopropylamine, methylethylamine, 2-methylthioethylamine, di-n-butylamine, dimethylamine, allylamine, cyclopantylamine, 2-(N-methyl-pyrrolidin-2-yl)ethylamine, tetrahydrofuranosyl-2-methylamine, piperidine, N-benzyl-4-aminopiperidine, aminomethylcyclopropane, cyclopropylamine, 3-methylpiperizine, 4-piperidin-1-ylpiperidine, cyclohexylamine, piperazine, 4-pyridin-2-ylpiperazine, 1-methylpiperazine, N-(2-methoxyphenyl)piperazine, N-pyrimidin-2-ylpiperazine, cycloheptylamine, p-trifluorobenzylamine, benzylamine, 3-imidazol-1-ylpropylamine, exo-2-aminonorborane, N-phenylethylenediamine, 1-methylbenzylamine, 3,4-(1,3-dioxolanyl)benzylamine, pyridin-2-ylmethylamine, pyridin-3-ylmethylamine, pyridin-4-ylmethylamine, thiophen-2-ylmethylamine, 3,3-dimethylbutylamine, o-methoxybenzylamine, 1-(3-aminopropyl)pyrrolidin-2-one, N-methylethylenediamine, m-methylbenzylamine, 3-methylbutylamine, 2-methylbutylamine, heptylamine, 3-butoxypropyamine, 3-isopropoxypropylamine, 2-morpholin-4-ylpropylamine, N1,N1-diethylethylenediamime, 2-ethylthioethylamine, 4-(2-aminoethyl)phenol, furfurylamine, 4-aminomethylpiperidine, 2-(2-aminoethyl)pyridine, 2-phenoxyethylamine, 2-aminoethylthiophene, p-methoxybenzylamine, 2-(N,N-dimethylamino)ethylamine, 1-amino-2-propanol, 5-methylfurfurylamine, 3-(dimethylamino)propylamine, o-methoxybenzylamine, 1-(3-aminopropyl)-2-pipecoline, hydrazine, 4-hydroxypiperidine, ethylenediamine, 1,4-diaminobutane, N-methylpropylamine, trans-1,4-diaminocyclohexane, 2,2,2-trifluoroethylamine, 3-chloropropylamine, 3-ethoxypropylamine, aminoacetaldehyde dimethyl acetal, 3-amino-1,2-propanediol, 1,3-diamino-2-hydroxypropane, 1-aminopyrrolidine, 2-(2-aminoethyl)-1-methylpyrrolidine, 3-methylpiperidine, 2-piperidine methanol, 3-piperidine methanol, 1-aminohomopiperidine, homopiperazine, 4-aminomorpholine, 3-bromobenzylamine, piperonylamine, 1,2,3,4-tetrahydroisoquinoline, L-proline methyl ester, 1-(2-pyridyl)piperazine, 4-(2-aminoethyl)morpholine, 1-(2-aminoethyl)piperidine, 3-aminopropipnitrile, 3-(aminomethyl)pyridine, 2-(aminomethyl)pyridine, thiomorpholine, 1,4-dioxa-8-azaspiro(4,5)-decane, 2-

hydroxylethylamine, 1-(2-aminoethyl)pyrrolidine, aminomethylcyclohexane, 2-hydroxymethylpyrrolidine, 3-amino-1,2-propanediol acetone ketal, N-(2-hydroxyethyl)piperazine, N-phenylethylenediamine, 4-amino-2,2,6,6-tetramethylpiperidine, N-(4-nitrophenyl)ethylenediamine, 1,2-diphenylethylamine, 1-(N,N-dimethylamino)-2-propylamine, 2-phenylpropylamine, 2-methylcyclopropylamine, 2-methylaziridine, aminomethylcyclopropane, 1-aminomethyl-2-methylcyclopropane, butten-3-ylamine, 3-methyl-buten-2-ylamine, 3-methyl-buten-3-ylamine, 4-aminomethyl-1-cyclohexene, 3-phenylallylamine, 2,2-dimethylethylenediamine, 3-ethylhexylamine, 3-(N,N-dimethylamino)-2,2-dimethylpropylamine, 2-methyl-N-aminopropylpiperidine, as well as other related aliphatic and aromatic primary and secondary amine, hydrazine, hydroxyamine, various amino acid, amino acid ester derivatives that are good nucleophiles to react with leaving groups on the scaffolds.

Exemplary Building Blocks for C-C Bond Formation

For Heck Reaction: 2-ethynylpyridine, 5-phenyl-1-pentyne, 4-(tert-butyl)phenylacetylene, phenylacetylene, 3-dibutylamino-1-propyne, phenyl propargyl ether, 5-chloro-1-pentyne, 3-diethylamino-1-propyne, 4-phenyl-1-butyne, 1-heptyne, 1-dimethylamino-2-propyne, 1-pentyne, 2-methyl-1-hexene, (triethylsilyl)acetylene, 3-phenyl-1-propyne, methyl propargyl ether, 3-cyclopentyl-1-propyne, 1-ethynylcyclohexene, 3-butyn-1-ol, styrene, vinylcyclohexane, 2-(tributylstannyl)furan, 2-(tributylstannyl)thiophene, tetraphenyltin, 3-cyclohexyl-1-propyne, 4-methoxyphenylacetylene, 4-(trifluoromethyl)phenyleneacetylene, 4-fluorophenylacetylene, 4-pentayn-1-ol, 4-methylphenylacetylene, 1-ethynylcyclopentanol, 3-methyl-1-propyne, 5-cyano-1-pentyne, cyclohexylethyne, 1-ethynylcyclohexene, 5-cyano-1-pentyne, 1-dimethylamino-2-propyne, N-methyl-N-propargylbenzylamine, 2-methyl-1-buten-3-yne, cyclopentylethyne, 4-nitrophenylacetylene, phenyl propargylsulfide, 4-methyl-1-pentyne, propargyl ethylsulfide, 2-prop-2-ynyloxybenzothiazole, 4-ethoxy-1-prop-2-ynyl-1,5-dihydro-2H-pyrrol-2-one, 6-methyl-5-(2-propynyl)-2-thioxo-2,3-dihydro-4(1H)-pyrimidinone and related end-alkenes and alkynes.

For Stille Reaction: tetraethyltin, 2-(tributylstannyl)pyridine, tributylstannyl-4-t-butylbenzene, ethynyltri-n-butyltin, vinyltri-n-butyltin, allyltri-n-butyltin, phenylethynyltri-n-butyltin, phenyltri-n-butyltin, (2-methoxy-2-cyclohexen-1-yl)tributyltin, 5,6-dihydro-2-

(tributylstannyl)-4H-pyran, tri-n-butyl(2-furanyl)tin, tri-n-butyl(2-thienyl)tin, tributyl(phenylethenyl)tin, 4-fluoro-(tri-n-butylstannyl)benzene, 5-fluoro-2-methoxy(tri-n-butylstannyl)benzene, 1-methyl-2-(tributylstannyl)-1H-pyrrole, 5-methyl-2-tributylstannylthiophene, 2-tributylstannylthiazole, 2-trybutylstannylpyrrazine, tributyl[3-(trifluoromethyl)phenyl]stannane and other related organic tin reagents.

For Suzuki Reaction: phenylboronic acid, 4-tolylboronic acid, 2-thiopheneboronic acid, thiophene-3-boronic acid, furan-2-boronic acid, cyclopentylboronic acid, 4-methylfuran-2-boronic acid, 3-hydroxyphenyl)boronic acid, 5-methylfuran-2-boronic acid, 3-cyanophenylboronic acid, 4-cyanophenylboronic acid, (5-formyl-3-furanyl)boronic acid, furan-3-boronic acid and other related organic boronic acids.

Exemplary Building Blocks ROH for Mitsunobu Reaction

1-Butanol, 4-nitrophenethyl alcohol, 4-chlorobenzyl alcohol, 1-propanol, 4-nitrobenzyl alcohol, 4-methylbenzyl alcohol, 2-butanol, benzyl alcohol, 2-methyl-1-propanol, crotyl alcohol, 2-norbornanemethanol, 2-methylcyclopropane-methanol, 3-buten-1-ol, neopentyl alcohol, cyclohexylmethanol, 4-trifluorobenzyl alcohol, 3-methyl-2-butem-1-ol, cyclopentanemethanol, 3-methyl-3-buten-1-ol, 4-methyl-1-pentanol, 3-chlorobenzyl alcohol, 3-cyclohexane-1-methanol, 3,3-dimethylbutanol, 3-trifluorobenzyl alcohol, cinnamyl alcohol, tetrahydrofurfuryl alcohol, ethanol, cyclopropyl alcohol, 1-methyl-3-piperidinemethanol, decahydro-2-naphthol, 9-decen-1-ol, 3-cyclopentyl-1-propanol, 1-methyl-2-pyrrolidineethanol, 3-methylbenzyl alcohol, 3-fluorobenzyl alcohol, 3-phenoxybenzyl alcohol, 4-isopropylbenzyl alcohol, 4-methoxybenzyl alcohol, 3,4-dimethoxybenzyl alcohol, 3,5-dimethylbenzyl alcohol, 4-benzyloxybenzyl alcohol, 2-phenylethanol, 4-fluorobenzyl alcohol, phenoxyethanol, benzyloxyethanol, 1-pentanol and 3-pentanol as well as aliphatic/aromatic/heterocyclic primary and secondary alcohols. Similar RSH derivatives have been used as building blocks for library synthesis.

All available aliphatic, aromatic and heterocyclic acyl chlorides, sulfonyl chlorides, isocyanates, thioisocyanates, carboxylic acids, amino acids, isocyanides, halogenated heterocycles for 2'- and 3'-NH_2 reactions.

BIOLOGICAL ASSAYS

The inventors discovered that various contemplated compounds exhibited significant antiviral effect, and especially significant antiviral effect against HCV in vitro and as NS5B inhibitors (data not shown). The assays used to measure the inhibition of HCV NS5B and other polymerases, in vitro cell-based HCV replication, BVDV, HIV, RSV, HRV, HBV, influenza, and cytotoxicity are described below.

Assay of De Novo RNA Synthesis Activity for HCV NS5B Polymerase

All the oligoribonucleosides were purchased from Oligo etc. and were gel-purified. All the chemical reagents were of highest purity possible. H_2O used in the assay was RNase and DNase free. [α-^{33}P]-CTP (Ci/mmol) was purchased from ICN Biochemicals or Perkin-Elmer.

A typical assay reaction was carried out at 23°C for one hour in a buffer containing 20 mM Tris, pH 8.0, 20 mM MgCl2, 10 mM KCl, 5 % Glycerol, 5 mM DTT and 0.5 mg/ml BSA. The template concentration was set at 10 mM and the enzyme concentration at 5 mM. The reaction was quenched by addition of a loading buffer (80% formamide, 100 mM EDTA, 50 mM Tris borate, 0.15% bromophenol blue and 0.15% of xylene cyanol) and heated to 70°C for 1 min prior to loading on a 1 X TBE polyacrylamide gel. Electrophoresis was performed in 1 X TBE at 3000 Volt. Gels were visualized and analyzed by using a PhosphorImager. Unless indicated otherwise, data are not shown for contemplated compounds.

HCV Replicon Assay

The replicon cells (Huh-7) contain replicating HCV replicon RNA, which was modified in the structural region (replacing the structural region with a neomycin resistance marker). Survival of the replicon cells under G418 selection relies on the replication of HCV RNA and subsequently expression of neomycin phosphoryltransferase. The ability of modified nucleoside libraries and compounds to suppress HCV RNA replication was determined using the Quantigene Assay Kit from Bayer. The assay measures the reduction of HCV RNA molecules in the treated cells. Replicon cells were incubated at 37°C for 3 days in the presence of nucleoside libraries and compounds before being harvested for detection. The

HCV subgenomic replicon cell line was provided by Dr. Bartenschlager. The assay protocol was modified based on literature procedure (V. Lohmann, F. Korner, J. O. Koch, U. Herian, L. Theilmann, R. Bartenschlager, *Science*, **1999**, *285*, 110-113). Unless indicated otherwise, data are not shown for contemplated compounds.

Assay for Inhibition of BVDV

Bovine viral diarrhea virus (BVDV) (strain NADL) was provided by Dr. Ruben Donis and propagated in MDBK cells (ATCC). The nucleoside libraries and compounds were tested utilizing the modified protocol (V. B. Vassilev, M. S. Collett, R. O. Donis, *J. Viol.* **1997**, *71*, 471-478; S. G. Bagginski, D. C. Pevear, M. Seipel, S. C. C. Sun, C. A. Benetatos, S. K. Chunduru, C. M. Rice, M. S. Collett, *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 7981-7986). Unless indicated otherwise, data are not shown for contemplated compounds.

Hepatitis B Virus (HBV) Assay

The in vitro anti-HBV activity of nucleoside libraries and compounds was tested based on the reported protocol (W. E. Delaney, 4^{th}, R. Edwards, D. Colledge, T. Shaw, J. Torresi, T. G. Miller, H. C. Isom, C. T. Bock, M. P. Manns, C. Trautwein, S. Locarnini, *Antimicrob. Agents Chemother.*, **2001**, *45*, 1705-1713; W. E. Delaney, 4^{th}, T. G. Miller, H. C. Isom, *Antimicrob. Agents Chemother.*, **1999**, *43*, 2017-2026; B. E. Korba, J. L. Gerin, *Antiviral Res.*, **1992**, *19*, 55-70). Unless indicated otherwise, data are not shown for contemplated compounds.

Human Immunodeficiency Virus (HIV) Assay

The in vitro HIV-1 activity of nucleoside libraries and compounds was tested utilizing the following modified protocol. Freshly isolated human PBMCs from healthy donors were infected with HIV-1 isolates for 3 hours. The cells were then washed three times to remove the viruses. The infected cells were plated into 96-well tissue culture plates and incubated for 7 days in the presence of serially diluted nucleoside analogues (with a medium change at day 4). A standardized HIV-1 p24 Elisa was performed to measure the extent of HIV replication in the presence of the compounds. (C. J. Petropoulos, N. T. Parkin, K. L. Limoli, Y. S. Lie, T. Wrin, W. Huang, H. Tian, D. Smith, G. A. Winslow, D. J. Capon, J. M. Whitcomb, *Antimicrob. Agents Chemother.*, **2000**, *44*, 920-928; Parkin, N. T., Y. S. Lie, N. Hellmann,

M. Markowitz., S. Bonhoeffer, D. D. Ho, C. J. Petropoulos, *J. Infect. Disease*, **1999**, *180*, 865-870). Unless indicated otherwise, data are not shown for contemplated compounds.

Human Rhinovirus (HRV) Assay

The in vitro activity of nucleoside libraries and compounds against HRV was tested based on the reported protocol (W.-M. Lee, W. Wang, R. Rueckert, *Virus Genes*, **1994**, *9*, 177-181; B. Sherry, R. Rueckert, *J. Virol.* **1985**, *53*, 137-143). Unless indicated otherwise, data are not shown for contemplated compounds.

Respiratory Syncytial Virus (RSV) Assay

The RSV activity of nucleoside libraries and compounds was tested based on the reported protocol. Respiratory syncytial virus (strain A-2) was purchased from ATCC and virus stock was obtained by propagating the virus in Hep-2 cells. (P. R. Wyde, L. R. Meyerson, B. E. Gilbert, *Drug Dev. Res.* **1993**, *28*, 467-472). Unless indicated otherwise, data are not shown for contemplated compounds.

Yellow Fever Virus (YFV) Assay

Yellow fever virus (vaccine strain 17-D) was purchased from ATCC (VR-1268) and the virus stock was obtained by infecting SW-13 cells from ATCC. The YFV activity of nucleoside libraries and compounds was tested utilizing the reported protocol (J. J. Schlesinger, S. Chapman, A. Nestorowicz, C. M. Rice, T. E. Ginocchio, T. J. Chambers, *J. Gen. Virol.* **1996**, *77*, 1277-1285). Unless indicated otherwise, data are not shown for contemplated compounds.

Influenza Virus Assay

Influenza virus (type A, A/PR/8/34) was produced by infecting pathogen-free fertilized chicken eggs. The antiviral assay was performed on Madin Darby canine kidney (MDCK) cells from ATCC based on the reported protocol (E. H. Nasser, A. K. Judd, A. Sanchez, D. Anastasion, D. J. Bucher, *J. Virol.* **1996**, *70*, 8639-8644). Unless indicated otherwise, data are not shown for contemplated compounds.

Cytotoxicity Assay

The cytotoxicity of nucleoside libraries and compounds was measured by the MTS cell based assay from Promega (CellTiter 96 Aqueous One Solution Cell Proliferation Assay). Unless indicated otherwise, data are not shown for contemplated compounds.

Consequently, it is contemplated that the compounds according to the inventive subject matter may be employed in pharmaceutical compositions to treat various viral diseases. In an especially preferred aspect of the inventive subject matter, the inventors contemplate a method of inhibiting replication of a virus in which one or more of the compounds according to the inventive subject matter are provided. In a further step, the virus is presented with the compound(s) at a concentration effective to inhibit replication of the virus. The term "presenting the virus with a compound" as used herein broadly refers to all manners in which the virus or viral component is incubated with the compound.

For example, where the virus or viral component (particularly including a viral RNA dependent RNA polymerase) is in an *in vitro* system, presentation may comprise admixing the medium in which the virus or viral component is disposed with the compound. In another example, where the virus or viral component is in a cell (either in a cell culture, or *in vivo* in a hepatocyte in an infected liver of a mammal) it is contemplated that the step of presenting may include administration of a pharmaceutical composition comprising contemplated compounds to the organism in which the virus or viral component is disposed. Suitable pharmaceutical compositions may include oral, parenteral, transdermal, and various other known pharmaceutical compositions. Hence, in an especially preferred aspect, the virus is an HCV virus and is disposed within a cell (which is preferably a hepatocyte in a liver infected with the virus).

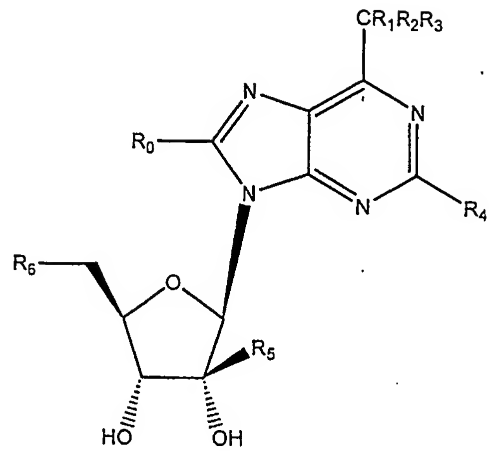
Thus, specific embodiments and applications of nucleoside analog libraries, library compounds and their use as antiviral agents have been disclosed. It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the appended claims. Moreover, in interpreting both the specification and the claims, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms "comprises" and "comprising" should be interpreted as referring to elements,

components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

CLAIMS

What is claimed is:

1. A compound according to Formula 1



Formula 1

wherein the sugar is in D- or L-configuration;

R_0 is H, or halogen, or optionally substituted alkyl, alkenyl, alkynyl, or aryl;

R_1, R_2, and R_3 are independently optionally substituted alkyl, alkenyl, alkynyl, aryl, or H, or where R_1 and R_2 are H, R_3 is alkyl-NR'R", alkyl-ONR'R", alkyl-NR'NR'R", alkyl-SR', alkyl-OR', or alkyl-CN;

R_4 is H or NH_2;

R_5 is optionally substituted alkyl, alkenyl, alkynyl, aryl, or CN, or CF_3;

R_6 is H, OH, phosphate, phosphonate, or boranophosphate; and

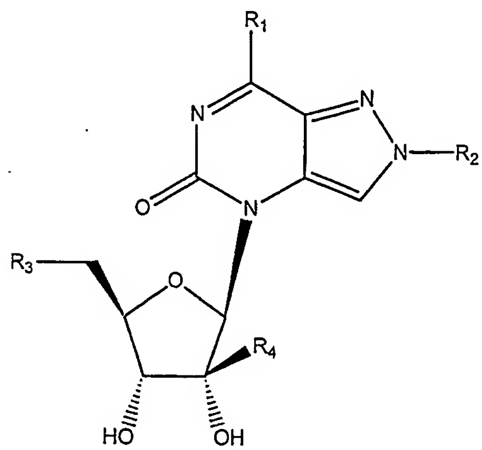
R' and R" are independently H, OH, or optionally substituted alkyl, alkenyl, alkynyl, aryl.

2. The compound according to claim 1 wherein R_5 is methyl.

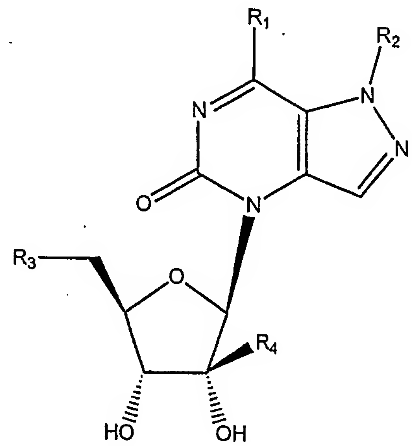
3. The compound according to claim 2 wherein R_6 is OH.

4. The compound according to claim 3 wherein R_0 is H, R_1 and R_2 are H, and wherein R_3 is alkyl-NR'R", alkyl-ONR'R", alkyl-NR'NR'R", alkyl-SR', alkyl-OR'.

5. A compound according to Formula 2A or Formula 2B



Formula 2A



Formula 2B

wherein the sugar is in D- or L-configuration;

R_1 is NR'R", ONR'R", NR'NR'R", SR', OR', or R';

R_2 is alkyl, acyl, or aryl;

R_3 is protected or unprotected OH, phosphate, phosphonate, or boranophosphate;

R_4 is optionally substituted alkyl, alkenyl, alkynyl, aryl, CN, or CF_3; and

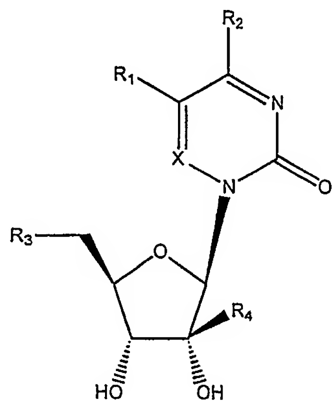
wherein R' and R" are independently H, OH, or optionally substituted alkyl, alkenyl, alkynyl, aryl.

6. The compound according to claim 5 wherein R_4 is methyl and wherein R_3 is OH.

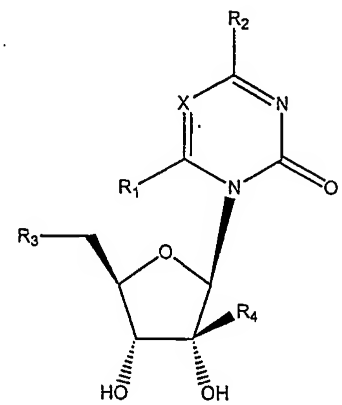
7. The compound according to claim 6 wherein R_1 is NR'R", ONR'R", or NR'NR'R".

8. The compound according to claim 6 wherein R_2 is alkyl.

9. A compound according to Formula 3A or Formula 3B



Formula 3A



Formula 3B

wherein the sugar is in D- or L-configuration;

X is N or CH;

R_1 is H, halogen, alkyl, or alkenyl;

R_2 is NR'R", ONR'R", NR'NR'R", SR', OR', or R';

R_3 is protected or unprotected OH, phosphate, phosphonate, or boranophosphate;

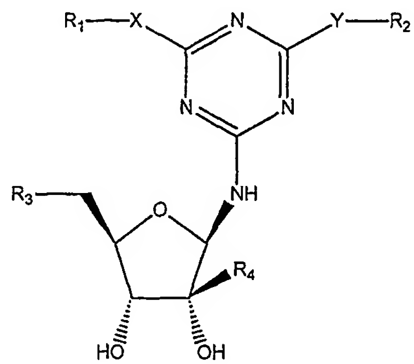
R_4 is optionally substituted alkyl, alkenyl, alkynyl, aryl, CN, or CF_3; and

wherein R' and R" are independently H, OH, or optionally substituted alkyl, alkenyl, alkynyl, aryl.

10. The compound according to claim 9 wherein R_4 is methyl and wherein R_3 is OH.

11. The compound according to claim 10 wherein R_1 is H and R_2 is NR'R", ONR'R", or NR'NR'R".

12. A compound according to Formula 4



Formula 4

wherein X and Y are independently null, NR', O or S;

R_1 and R_2 are independently NR'R" or H, or optionally substituted alkyl, alkenyl, alkynyl, or aryl;

R_3 is protected or unprotected OH, phosphate, phosphonate, or boranophosphate;

R_4 is optionally substituted alkyl, alkenyl, alkynyl, aryl, or CN or CF_3; and

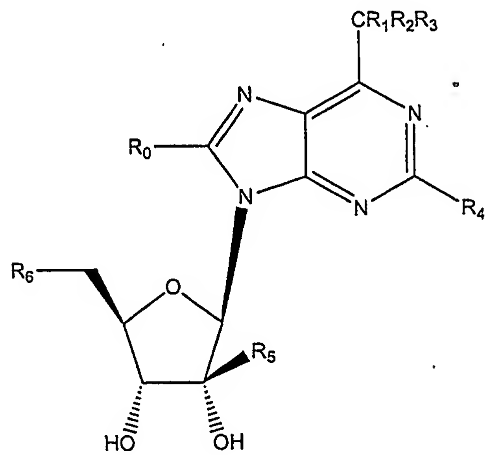
R' and R" are independently H or OH, or optionally substituted alkyl, alkenyl, alkynyl, or aryl.

13. The compound according to claim 12 wherein R_3 is OH and R_4 is CH_3.

14. The compound according to claim 13 wherein X and Y are NR'.

AMENDED CLAIMS
[received by the International Bureau on 02 July 2003 (02.07.03);
original claims 1-14 replaced by new claims 1-14.]

1. A compound according to Formula 1



Formula 1

wherein the sugar is in D- or L-configuration;

R_0 is H, or halogen, or optionally substituted alkyl, alkenyl, alkynyl, or aryl;

R_1, R_2, and R_3 are independently optionally substituted alkyl, alkenyl, alkynyl, aryl, or H, or where R_1 and R_2 are H, R_3 is alkyl-NR'R", alkyl-ONR'R", alkyl-NR'NR'R", alkyl-SR', alkyl-OR', or alkyl-CN;

R_4 is H or NH_2;

R_5 is optionally substituted alkyl, alkenyl, alkynyl, aryl, or CN, or CF_3;

R_6 is H, OH, phosphate, phosphonate, or boranophosphate; and

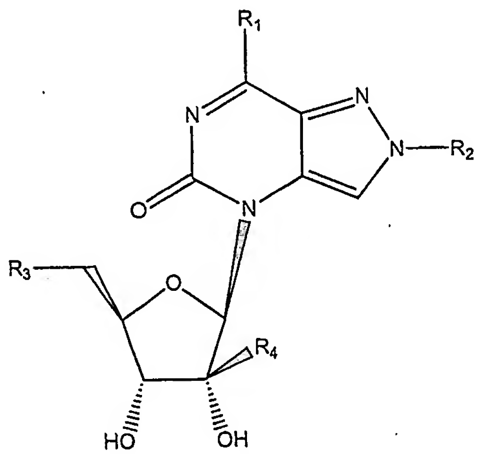
wherein R' and R" are independently H, OH, or optionally substituted alkyl, alkenyl, alkynyl, aryl.

2. The compound according to claim 1 wherein R_5 is methyl.

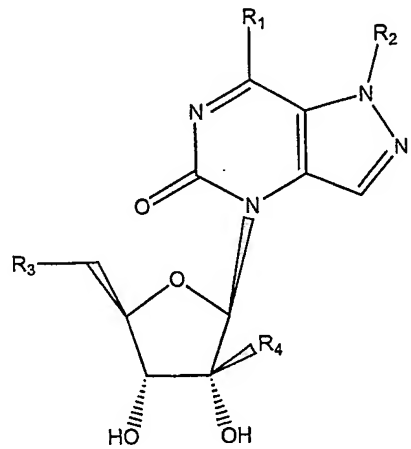
3. The compound according to claim 2 wherein R_6 is OH.

4. The compound according to claim 3 wherein R_0 is H, R_1 and R_2 are H, and wherein R_3 is alkyl-NR'R", alkyl-ONR'R", alkyl-NR'NR'R", alkyl-SR', alkyl-OR'.

5. A compound according to Formula 2A or Formula 2B



Formula 2A



Formula 2B

wherein the sugar is in D- or L-configuration;

R_1 is NR'R", ONR'R", NR'NR'R", SR', OR', or R';

R_2 is selected from the group consisting of alkyl, acyl, and aryl;

R_3 is protected or unprotected OH, phosphate, phosphonate, or boranophosphate;

R_4 is optionally substituted alkyl, alkenyl, alkynyl, aryl, CN, or CF_3; and

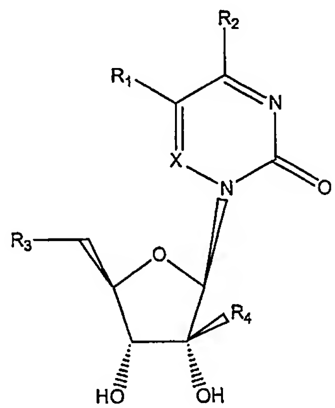
wherein R' and R" are independently H, OH, or optionally substituted alkyl, alkenyl, alkynyl, aryl.

6. The compound according to claim 5 wherein R_4 is methyl and wherein R_3 is OH.

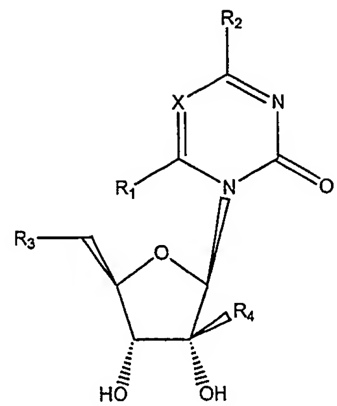
7. The compound according to claim 6 wherein R_1 is NR'R", ONR'R", or NR'NR'R".

8. The compound according to claim 6 wherein R_2 is alkyl.

9. A compound according to Formula 3A or Formula 3B



Formula 3A



Formula 3B

wherein the sugar is in D- or L-configuration;

X is N or CH;

R_1 is H, halogen, alkyl, or alkenyl;

R_2 is NR'R", ONR'R", NR'NR'R", SR', OR', or R';

R_3 is protected or unprotected OH, phosphate, phosphonate, or boranophosphate;

R_4 is optionally substituted alkyl, alkenyl, alkynyl, aryl, CN, or CF_3; and

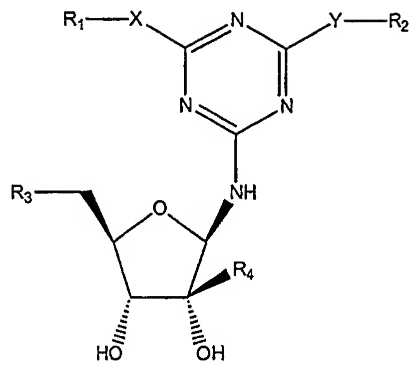
wherein R' and R" are independently H, OH, or optionally substituted alkyl, alkenyl, alkynyl, aryl; and

with the proviso that where in compounds according to Formula 3A X is CH and R_2 is NH_2, then R_1 is not H, Halogen, or alkyl.

10. The compound according to claim 9 wherein R_4 is methyl and wherein R_3 is OH.

11. The compound according to claim 10 wherein R_1 is H and R_2 is NR'R", ONR'R", or NR'NR'R".

12. A compound according to Formula 4



Formula 4

wherein X and Y are independently null, NR', O or S;

R_1 and R_2 are independently NR'R" or H, or optionally substituted alkyl, alkenyl, alkynyl, or aryl;

R_3 is protected or unprotected OH, phosphate, phosphonate, or boranophosphate;

R_4 is optionally substituted alkyl, alkenyl, alkynyl, aryl, or CN or CF_3; and

wherein R' and R" are independently H or OH, or optionally substituted alkyl, alkenyl, alkynyl, or aryl.

13. The compound according to claim 12 wherein R_3 is OH and R_4 is CH_3.

14. The compound according to claim 13 wherein X and Y are NR'.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/01557

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07H 19/00; A01N 43/04; A61K, 31/70
US CL : 536/26.1, 26.11, 26.12, 26.13, 27.21, 27.6, 27.62, 28.5; 514/45, 46, 47, 48, 49
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 536/26.1, 26.11, 26.12, 26.13, 27.21, 27.6, 27.62, 28.5; 514/45, 46, 47, 48, 49

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST, STN (REGISTRY, CAPLUS, MEDLINE, BIOSIS)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/90121 A2 (NOVIRIO PHARMACEUTICALS LIMITED et al.) 29 November 2001 (29.11.2001).	1-14
X	WO 01/92282 A2 (NOVIRIO PHARMACEUTICALS LIMITED et al.) 6 December 2001 (06.12.2001).	1-14
X, P	WO 02/057425 A2 (MERCK & CO., INC. et al.) 25 July 2002 (25.07.2002).	1-14
X, P	WO 02/18404 A2 (F. HOFFMANN-LA ROCHE AG) 7 March 2002 (07.03.2002).	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 April 2003 (21.04.2003)	02 MAY 2003
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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

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(54) Title: NUCLEOTIDE MIMICS AND THEIR PRODRUGS

(57) Abstract: The present invention relates to nucleoside disphosphate mimics and nucleoside triphosphate mimics, which contain disphosphate or triphosphate moiety mimics and optionally sugar-modifications and/or base-modifications. The nucleotide mimics of the present invention, in a form of a pharmaceutically acceptable salt, a pharmaceutically acceptable prodrug, or a pharmaceutical formulation, are useful as antiviral, antimicrobial, and anticancer agents. The present invention provides a method for the treatment of viral infections, microbial infections, and proliferative disorders. The present invention also relates to pharmaceutical compositions comprising the compounds of the present invention optionally in combination with other pharmaceutically active agents.

NUCLEOTIDE MIMICS AND THEIR PRODRUGS

BACKGROUND OF THE INVENTION

This application asserts priority to U.S. provisional application Serial No. 60/360,699 filed February 28, 2002 and U.S. provisional application Serial No. 60/360,915 filed February 28, 2002, which are incorporated herein by reference in their entirety.

[0001] Viral infections are a major threat to human health and account for many serious infectious diseases. Hepatitis C virus (HCV), a major cause of viral hepatitis, infects more than 200 million people worldwide. Current treatment for HCV infection is restricted to immunotherapy with interferon-α, alone or in combination with Ribavirin, a nucleoside analog. This treatment is effective in only about half of the patients. Hepatitis B virus (HBV) acutely infects almost a third of the world's human population, and about 5% of the infected are chronic carriers of the virus. Chronic HBV infection causes liver damage that frequently progresses to cirrhosis and liver cancer later in life. Despite the availability and widespread use of effective chemotherapy and vaccines, the number of chronic carriers approaches 400 million worldwide.

[0002] Human immunodeficiency virus (HIV) causes progressive degeneration of the immune system, leading to the development of AIDS. A number of drugs have been used clinically, including HIV nucleoside and non-nucleoside reverse transcriptase inhibitors and protease inhibitors. Currently, combination therapies are the accepted standard of treatment of AIDS in order to reduce viral load and the emergence of drug resistance. Despite progress in the development of anti-HIV drugs, AIDS is still one of the leading epidemic diseases. Therefore, there is still an urgent need for new, more effective HCV, HBV, and HIV drugs. The treatment of viral infections caused by other viruses such as HSV, CMV, influenza viruses, West Nile virus, small pox, EBV, VZV, and RSV also needs better medicines.

[0003] Bacterial infections long have been the sources of many infectious diseases. The widespread use of antibiotics results in the emergence of drug-resistant life-threatening bacteria. Fungal infections are another type of infectious disease, some of which also can be life-threatening. There is an increasing demand for the treatment of bacterial and fungal infections. Antimicrobial drugs based on new mechanisms of action and new chemical classes are especially important.

[0004] Cellular proliferative disorders are responsible for numerous dieases resulting in major morbidity and mortality and have been intensively investigated for decades. Cancer now is the second leading cause of death in the United States, and over 500,000 people die annually

from this proliferative disorder. All of the various cells types of the body can be transformed into benign or malignant tumor cells. Transformation of normal cells into cancer cells is a complex process and, thus far, is not fully understood. Treatment of cancer normally consists of surgery, radiation, and chemotherapy. While chemotherapy can be used to treat all types of cancer, surgery and radiation therapy are limited to certain cancers at various stages of growth at certain sites of the body. There are a number of anticancer drugs widely used clinically. Among them are alkylating agents such, as cisplatin, and antimetabolites, such as 5-Fluorouracil, Gemcitabine, Cytarabine, Fludarabine, and Cladarabine. Although surgery, radiation, and chemotherapies are available to treat cancer patients, there is no cure for cancer at the present time. Cancer research is still one of the most important endeavors in medical and pharmaceutical organizations.

[0005] Nucleoside analogs have been used clinically for the treatment of viral infections and proliferative disorders for decades. Most of the nucleoside drugs are classified as antimetabolites. After they enter cells, nucleoside analogs are successively phosphorylated to nucleoside 5'-monophosphates, 5'-diphosphates, and 5'-triphosphates. In most cases, nucleoside triphosphates, e.g. 3'-azido-3'-deoxythymidine (AZT, an anti-HIV drug) triphosphate and arabinosylcytosine (Cytarabine, an anticancer drug) triphosphate, are the chemical entities that inhibit DNA or RNA synthesis, either through a competitive inhibition of polymerases or through incorporation of modified nucleotides into DNA or RNA sequences. Nucleosides may act also as their diphosphates. For instance, 2'-deoxy-2',2'-difluorocytidine (Gemcitabine, an anticancer drug) 5'-diphosphate has been shown to inhibit human ribonucleotide reductase. Current clinically-used nucleoside drugs primarily depend on cellular activation by nucleoside kinases (nucleoside to nucleotide) and nucleotide kinases (nucleotides to di- and tri-nucleotides). Efficient phosphorylation at each step is required for a nucleoside to be an effective drug.

[0006] In order to overcome the usual deficiencies of cellular phosphorylation of unnatural nucleosides, nucleotides, themselves, have been considered as antimetabolite drugs. However, the multiply-charged nucleotides do not effectively penetrate cell membranes and often are hydrolyzed by certain extracellular enzymes. In the last two decades, nucleoside mono-phosphate prodrugs have been intensively investigated as an alternative drug form (Wagner *et al., Med. Res. Rev.* **2000**, 20, 417-451; Jones *et al., Antiviral Res.* **1995**, 27, 1-17; Perigaud *et al. Adv. in Antiviral Drug Des.* **1995**, 2, 147-172; Huynh-Dinh, *Curr. Opin. Invest. Drugs*, **1993**, 2, 905-915). It was hoped that nucleoside mono-phosphate prodrugs, which mask

the negative charges on the phosphate by reversible chemical modifications, now being much more lipophilic, would transverse cell membranes and liberate the nucleoside mono-phosphate intracellularly. Cleavage of the prodrug moiety from the nucleoside mono-phosphate would proceed enzymatically via a variety of ubiquitous, non-specific enzymes, like esterases or hydrolytically. Having now bypassed the first kinase step, which is often the most difficult of the three steps with unnatural nucleoside, higher concentration of the required, active species, the nucleoside tri-phosphate was expected. Progress in the area of nucleotide phosphate prodrugs has been made. For instance, certain phosphate prodrugs of anti-HIV nucleosides have been explored for their use as antiviral drugs. The di-and tri-phosphates of 3'-deoxy-3'-azidothymidine (AZT) and 2',3'-didehydro-2',3'-dideoxythymidine (D4T) were converted to their acyl prodrugs (Bonnaffe *et al. J. Org. Chem.* **1996**, 61, 895-902). AZT di- and tri-phosphate prodrugs demonstrated similar inhibition of HIV-infected cells as AZT itself, while the corresponding D4T di-and tri-phosphate prodrugs exhibited lower, but still significant anti-HIV activity. Since the acylphosphate moiety of the prodrugs is sensitive to chemical hydrolysis, it is assumed that the prodrugs had been converted to AZT and D4T before they enter cells. Phospholipids also have been as the masking moiety of nucleoside mono- and di-phosphates. AZT di-phosphate tethered with a thioether lipid showed potent inhibition of HIV-infected CEM cells (Hong *et al. J. Med. Chem.* **1996**, 39, 1771-1777). Other lipid-tethered nucleoside di- and tri-phosphates also have been studied (Hostetler *et al., J. Biol. Chem.* **1990**, 265, 6112-6117). Some antitumor nucleosides were also converted to the corresponding nucleotide prodrugs aimed at enhancing antitumor activities. Treatment with lipid-tethered Ara-C di-phosphates demonstrated longer life-span of p388-infected mice than that with Ara-C itself (Hong *et al., J. Med. Chem.* **1986**, 29, 2038-2044; **1990**, 33, 1380-1386). 8-Aza-2-deoxyadenosine and 8-bromo-2-deoxyadenosine, two weakly cytotoxic agents, were converted to their 5'-bis(pivaloxymethyl)phosphate prodrugs, which exhibited significantly improved cytotoxicity (Rose *et al., J. Med. Chem.* **2002,** published on web).

[0007] Although the prodrugs of nucleotides bearing natural phosphates exhibited certain *in vitro* and *in vivo* activities, several major obstacles remain to be overcome. The most obvious barrier is the inherent instability of the natural phosphates to cellular enzymes. Nucleotide prodrugs may, in certain cases, deliver negatively-charged nucleotides into cells better than the parent nucleotides, but are not significantly stabile towards enzymatic and hydrolytic degradation. In addition, nucleoside phosphates bearing natural phosphates when

released from their prodrugs intracellularly, may not be anabolized to the required active species (nucleoside di- or tri- phosphates), but may be catabolized back to the inactive parent nucleoside, which is resistant to phosphoylation. In several cases, not only is the active species not formed in sufficient concentrations to elicit effective therapeutic effects, but instead, an intermediate nucleoside phosphate that is formed may be a toxic species. As a case in point, AZT mono-phosphate accumulates in cells because the nucleoside mono-phosphate is a poor substrate for thymidylate kinase and is thought to be responsible for cellular toxicity.

[0008] In order to stabilize nucleotides, several nucleoside phosphates bearing di-phosphate or tri-phosphate mimics have been prepared and some of them have been evaluated various biological assays. Many nucleotide mimics or their biological use have been disclosed (Eckstein *et al.* U. S. Patent No. 3,846,402 issued November 1974; Horwitz *et al.*, U.S. Patent No. 4,266,048 issued May 1981; Schinazi *et al.*, U.S. Patent No. 5,118,672 issued June 1992; Ingels *et al.*, U.S. Patent No. 5,721,219 February 1998; Bottaro, *et al.*, U.S. Patent No. 6,303,774 October 2001; Boucher *et al.* U.S. Patent No. 6,143,279 issued November 2000; Johansson, EP0357571, 7/1990; Lebeau *et al.* WO9600733, 1/1996; Vladimirovich *et al.*, WO9820017, 5/1998; Watanabe, WO0179246, 4/2001; Yerxa *et al.*, WO0145691, 6/2001; Peterson, WO0187913, 11/2001). The early work in the chemistry and biological evaluations of nucleotide mimics have been reviewed (Scheit, K.H., *Nucleotide Analogs,* John Wiley & Sons, New York, (1980); Engel, R., *Chem. Rev.* **1977**, 77, 349-467; Yount, R.G., *Adv. in Enzymol.* **1975**, 43, 1-56).

[0009] One type of nucleoside di- and tri-phosphate mimic has modifications at the bridging positions of nucleoside diphosphates and triphosphates (Yount *et al., Biochemistry* **1971**, 10, 2484-2489; Ma *et al., J. Med. Chem.* **1992,** 35, 1938-1941; Ma *et al., Bioorg. Chem.* **1989,** 17, 194-206; Li *et al., Bioorg. Chem.* **1996**, 24, 251-261; Trowbridge *et al., J. Am. Chem. Soc.* **1972**, 94, 3816-3824; Stock, *J. Org. Chem.* **1979**, 44, 3997-4000; Blackburn *et al., J. Chem. Soc. Chem. Comm.* **1981**, 1188-1190; Shipitsin *et al., J. Chem. Soc. Perkin Trans 1,* **1999**, 1039-1050; Arabshahi *et al., Biochemistry*, **1990**, 29, 6820-6826; Yanachkov *et al., Nucleosides Nucleotides* **1994,** 13, 339-350). Among these phosphate mimics are the β,γ-imidotriphosphates, β,γ-methylimidotriphosphates, α,β-imidotriphosphates, α,β:β,γ-diimidotriphosphates, α,β-methylenetriphosphates, β,γ-methylenetriphosphates, α,β:β,γ-bismethylenetriphosphates, β,γ-dihalomethylenetriphosphates, α,β-

dihalomethylenetriphosphates, β,γ-halomethylenetriphosphates, and α,β-halomethylenetriphosphates. These phosphate mimics usually enhance the stability of the nucleotide towards hydrolysis by cellular enzymes. Methylene and halomethylenes render the nucleoside di- and tri-phosphate mimics considerable more stable to both chemical and enzymatic hydrolysis.

[0010] Another type of nucleoside di-phosphate and tri-phosphate mimic is the substitution of one or more phosphate non-bridging oxygen with other heteroatoms or functional group (Ludwig *et al., J. Org. Chem.* **1991**, 56, 1777-1783; Dineva, *Nucleosides Nucleotides* **1996**, 15, 1459-1467; Dyatkina *et al. Nucleosides Nucleotides* **1995**, 14, 91-103; He *et al., J. Org. Chem.* **1998**, 63, 5769-5773; He *et al., Nucleic Acids Res.* **1999**, 27, 1788-1794; Meyer *et al., EMBO* **2000**, 19, 3520-3529; Arzumanov *et al., J. boil. Chem.* **1996**, 271, 24389-24394). Among these phosphate mimics are α-*O*-alkyltriphosphate, α-*O*-aryltriphosphate, α-*P*-alkyltriphosphate, α-*P*-aryltriphosphate, α-P-alkylaminotriphosphate, α-*P*-thiotriphosphate, α-*P*-boranotriphosphate, γ-*O*-alkyltriphosphate, γ-O-aryltriphosphate, γ-*P*-alkyltriphosphate, γ-*P*-aryltriphosphate, γ-*P*-alkylaminotriphosphate, γ-*P*-thiotriphosphate. This type of modification on α– or β-phosphorus usually produces diastereomers due to the formation of a chiral phosphorus center. These nucleoside phosphate mimics generally are more stable to cellular nucleases than natural nucleoside phosphates.

[0011] Other nucleoside di- and tri-phosphate mimics include modifications at the 5'-position of nucleosides. For instance, 3'-azido'-3',5'-dideoxy-5'-methylenethymidine 5'-C-triphosphate in which the 5'-oxygen is replaced with methylene was synthesized and evaluated for anti-HIV activity (Freeman *et al., J. Med. Chem.* **1992**, 35, 3192-3196). The nucleotide mimics in which the 5'-oxygen is replaced by sulfur or amino also were reported (Trowbridge *et al., J. Am. Chem. Soc.* **1972**, 94, 3816-3824; Letsinger *et al., J. Am. Chem. Soc.* **1972**, 94, 292-293; Scheit *et al., J. Carbohydr. Nucleosides Nucleotides* **1974**, 1, 485-490). There are very few nucleotide mimics comprising combinations of two or more phosphate modifications. So far, only nucleotide mimics containing α,β:β,γ-diimidotriphosphate, α,β:β,γ-bismethylenetriphosphate, and α-*P*-borano-α-*P*-thiotriphosphate were reported, which contain two modifications each. The parent nucleosides for the preparation of nucleotide mimics in the reported work are generally selected from natural nucleosides and a few well known antiviral nucleosides such as AZT, D4T, and 3'-deoxythymidine.

[0012] Some of these nucleotide mimics have been evaluated for their biological activity. AZT 5'-α-*P*-boranotriphosphate and D4T 5'-α-*P*-boranotriphosphate exhibited very potent inhibition of HIV reverse transcriptase (RT) with Ki values in the low nM range in assays using homopolymer templates. AZT 5'-β,γ-difluoromethylenetriphosphate and AZT 5'-β,γ-imidotriphosphates also exhibited significant inhibition of DNA polymerase or HIV RT. The negatively-charged nucleotide mimics are not likely to be taken up intact by cells, and no meaningful cell-based antiviral data for di- and tri-nucleotide mimics has ever been published. These nucleotide mimics that are active in cell free biochemical assays contain only one modification each, either at the triphosphate bridging position or simply as a substitution of a phosphate oxygen and, thus, are ready substrates for enzymatic hydrolysis. Therefore, the mimics rapidly are degraded extracellularly to provide the parent nucleoside. Any biological activity would result from the parent nucleoside being taken into cells and anabolized to an active nucleotide.

[0013] Nucleotide di- and tri-phosphate mimics that are resistant to cellular enzymes and demonstrate significant biological activities have not been disclosed. The several known nucleotide mimics are constructed from natural nucleosides, such as adenosine, or from known biologically active nucleosides, such as AZT. Furthermore, it is essential that novel nucleoside di- and tri-phosphate mimics that are resistant to enzymatic degradation possess one or more prodrugs to allow effective intracellular transport. Nucleoside di- and tri-phosphate mimics with attached prodrugs have not been disclosed in the literature.

Summary of the Invention

[0014] There is a need for effective nucleotide drugs, which do not require anabolic enzymatic activation. In one aspect, this invention overcomes the need for anabolic enzymatic activation of nucleosides to active nucleotide species by providing the nucleosides as their di- and tri-phosphates. In this case, the di- and tri-phosphate moieties have been modified one or more times to provide novel nucleotide mimics that are resistant to degrading enzymes.

[0015] Thus, in this aspect of the invention novel nucleotide mimics are provided that contain a modified di- or tri-phosphate mimic that is stable to chemical and enzymatic degradation via hydrolysis, phosphorolysis and other possible reaction types. Furthermore, in another aspect of the invention, the novel nucleoside di- and tri-phosphates are converted into prodrugs to enhance drug absorption and/or drug delivery into cells. Another aspect of the

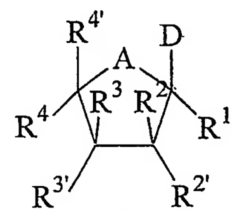
present invention is to provide a combination of the first two aspects and provide prodrugs of novel nucleotide mimics to enhance drug absorption and/or drug delivery into cells.

[0016] Another aspect of the present invention is to provide novel nucleotide mimics that are a combination of a novel nucleoside and a di- or tri-phosphate mimic.

[0017] An additional aspect of the present invention is to provide novel nucleotide mimics as a composition for therapeutic use for treatment of viral infections, microbial infections, parasite infections and cellular proliferative disorders.

[0018] A further aspect of the present invention is to provide a method for treatment of viral infections, microbial infections, parasite infections and proliferative disorders comprising administrating a nucleotide mimic or its prodrug of the present invention.

[0019] Such compounds of the invention include prodrugs (*i.e.,* one of X^7-10^x must be the prodrug substituent R*) such as a compound of Formula **(I)** which may be a D- or L-nucleotide:



(I)

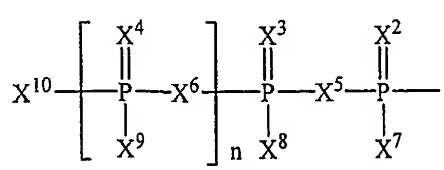
wherein

A is O, S, CY_2, NH or NR;

$R^{4'}$ is –L-R^5

L is selected from the group consisting of O, S, NH, NR, CY_2O, CY_2S, CY_2NH, CY_2, CY_2CY_2, CY_2OCY_2, CY_2SCY_2, and CY_2NHCY_2, wherein Y is selected from the group consisting of H, F, Cl, Br, alkyl, alkenyl, and alkynyl, wherein alkyl, alkenyl, and alkynyl may each optionally contain one or more heteroatoms;

R^5 is a di- or tri-phosphate moiety of Formula **(II)**:



(II)

X^2, X^3, and X^4 are selected independently from the group consisting of O, S, Se, NH and NR;

X^5 and X^6 are selected independently from the group consisting of O, S, Se, O_2, CY_2CO, CHOH, $C(OH)_2$, CH_2O, CH_2CH_2, CH_2CHNH_2, $CH_2CH_2CHNH_2$, CY_2OCY_2, CY_2, CRY, CY_2CY_2, CHR, CC, HC=CH, NH, NR, NOH, NOR, NNH_2, and NNHR;

X^7, X^8, X^9, and X^{10} are selected independently from the group consisting of H, F, OH, SH, NH_2, NHOH, NHOR, $NHNH_2$, NHNHR, CN, N_3, $^-BH_3M^+$, R, OR, SR, SeH, SeR, NHR, NR_2, and R*, wherein R* is a prodrug substituent;

wherein at least one of X^7, X^8, X^9 and X^{10} is R*;

R* may be conjugated to one or more X^7-X^{10} positions

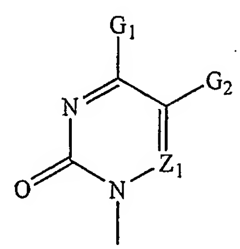
R is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, acyl, and aralkyl, each optionally containing one or more heteroatoms;

M^+ is a cation;

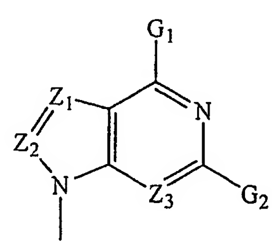
n is 0 or 1;

R^1, R^2, $R^{2'}$, R^3, $R^{3'}$, and R^4 are selected independently from the group consisting of H, F, Cl, Br, OH, SH, NH_2, NHOH, N_3, NO_2, CHO, COOH, CN, $CONH_2$, COOR, R, OR, SR, SSR, NHR, and NR_2; alternatively, R^2 and $R^{2'}$ together and R^3 and $R^{3'}$ together independently are =O, =S, or =J-Q, where J is N, CH, CF, CCl, or CBr and Q is H, F, Cl, Br, N_3, or R;

D is a nucleoside base of Formula **(III)** or **(IV)**:



(III)



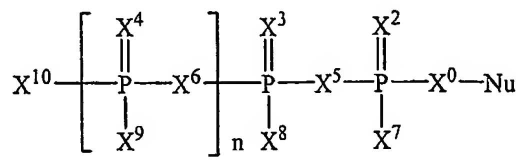
(IV)

Z^1, Z^2, and Z^3 are independently N, CH or C-G^3; and

G^1, G^2, and G^3 are selected independently from the group consisting of H, F, Cl, Br, I, OH, SH, NH_2, NHOH, $NHNH_2$, N_3, NO, NO_2, CHO, COOH, CN, $CONH_2$, CONHR, $C(S)NH_2$,

C(S)NHR, COOR, R, OR, SR, NHR, and NR_2; when two of G^3 are present on a molecule they may be same as or different from each other.

[0020] Such prodrugs of the invention also include (*i.e.*, one of X^7-X^{10} must be the prodrug substituent R*) also include compound of Formula **(XVI)**:



(XVI)

wherein X^0, X^5 and X^6 are selected independently from the group consisting of O, S, NH, and CY_2;

wherein Y is selected from the group consisting of H, F, Cl, Br, alkyl, alkenyl, and alkynyl, wherein alkyl, alkenyl, and alkynyl may each optionally contain one or more heteroatoms;

wherein X^2, X^3, and X^4 are selected independently from the group consisting of O, S, and Se;

wherein X^7, X^8, X^9, and X^{10} are selected independently from the group consisting of H, F, OH, SH, NH_2, NHOH, CN, N_3, $^-BH_3M^+$, R, R*, OR, SR, SeH, SeR, NHR, and NR_2;

wherein at least one of X^7, X^8, X^9 and X^{10} is R*;

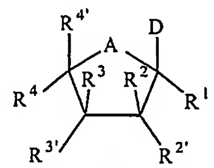
wherein n is 0 or 1;

wherein Nu is selected from the group consisting of natural nucleosides, sugar-modified nucleosides, base-modified nucleosides, and nucleosides with both sugar and base modifications;

wherein Nu is linked to X^0 through CH_2 of the sugar moiety of Nu; and

R is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, acyl, and aralkyl each optionally containing one or more heteroatoms.

[0021] In addition, nucleotide mimics of the invention (*i.e.*, X^7-X^{10} do not require the presence of a prodrug substituent R*) include compounds of Formula (I) which may be a D- or L-nucleotide:



(I)

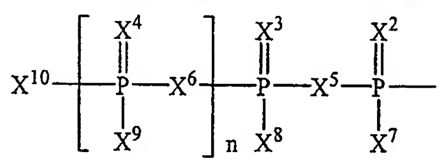
wherein

A is O, S, CY_2, NH or NR;

$R^{4'}$ is –L-R^5

L is selected from the group consisting of O, S, NH, NR, CY_2O, CY_2S, CY_2NH, CY_2, CY_2CY_2, CY_2OCY_2, CY_2SCY_2, and CY_2NHCY_2, CY_2SCY_2, and wherein Y is selected from the group consisting of H, F, Cl, Br, alkyl, alkenyl, and alkynyl, wherein alkyl, alkenyl, and alkynyl optionally contain one or more heteroatoms;

R^5 is a di- or tri-phosphate moiety of Formula (II):



(II)

X^2, X^3, and X^4 are selected independently from the group consisting of O, S, Se, NH and NR;

X^5 and X^6 are selected independently from the group consisting of O, S, Se, O_2, CY_2CO, CHOH, $C(OH)_2$, CH_2O, CH_2CH_2, CH_2CHNH_2, $CH_2CH_2CHNH_2$, CY_2OCY_2, CY_2, CRY, CY_2CY_2, CHR, CC, HC=CH, NH, NR, NOH, NOR, NNH_2, and NNHR;

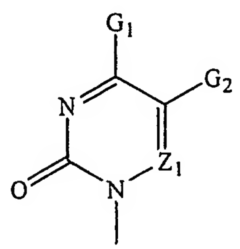
X^7, X^8, X^9, and X^{10} are selected independently from the group consisting of H, F, OH, SH, NH_2, NHOH, NHOR, $NHNH_2$, NHNHR,CN, N_3, $^-BH_3M^+$, R, OR, SR, SeH, SeR, NHR, and NR_2.

wherein n is 0 or 1;

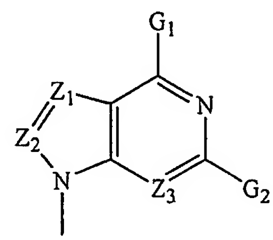
R^1, R^2, $R^{2'}$, R^3, $R^{3'}$, and R^4 are selected independently from the group consisting of H, F, Cl, Br, OH, SH, NH_2, NHOH, N_3, NO_2, CHO, COOH, CN, $CONH_2$, COOR, R, OR,

SR, SSR, NHR, and NR_2; alternatively, R^2 and $R^{2'}$ together and R^3 and $R^{3'}$ together independently are =O, =S, or =J-Q, where J is N, CH, CF, CCl, or CBr and Q is H, F, Cl, Br, N_3, or R;

D is a nucleoside base of Formula (**III**) or (**IV**):



(**III**)



(**IV**)

Z^1, Z^2, and Z^3 are independently N, CH or C-G^3;

G^1, G^2, and G^3 are selected independently from the group consisting of H, F, Cl, Br, I, OH, SH, NH_2, NHOH, $NHNH_2$, N_3, NO, NO_2, CHO, COOH, CN, $CONH_2$, CONHR, C(S)NH_2, C(S)NHR, COOR, R, OR, SR, NHR, and NR_2; when two of G^3 are present on a molecule they may be same as or different from each other; and

R is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, acyl, and aralkyl each optionally containing one or more heteroatoms;

with provisos that:

(1) when L is CH_2O and is linked to P through O and if one or more of X^7-X^{10} is selected from -BH_3M^+, SH, F, SR, R, OR, NH_2, NHR, and NR_2, then X^2-X^6 and the rest of X^7-X^{10} are not all O or OH; or when L is CH_2O and is linked to P through O and if one or more of X^7- X^{10} is selected from SH, F, SR, R, OR, NH_2, NHR, and NR_2, then X^2-X^6 and the rest of X^7-X^{10} are not all O or OH;

(2) when L is CH_2O and is linked to P through O and X^7- X^{10} are OH or OR, not all X^2-X^4 are O;

(3) when L is CH_2O and is linked to P through O and if one or more of X^2-X^4 is selected from S, NH, NR, NH, then X^5-X^{10} are not all O or OH, and one of X^5-X^{10} cannot be S when the rest of X^5-X^{10} are O or OH; or when L is CH_2O and is linked to P through O and if one of X^2-X^4 is selected from S, NH, NR, NH, then X^5-X^{10} are not all O or OH, and one of X^5-

X^{10} cannot be S when the rest of X^5-X^{10} are O or OH; or when L is CH_2O and is linked to P through O and if two of X^2-X^4 is selected from S, NH, NR, NH, then X^5-X^{10} are not all O or OH, and one of X^5-X^{10} cannot be S when the rest of X^5-X^{10} are O or OH

(4) when L is CH_2O and is linked to P through O and if the following X^6-X^5 pairs are CY_2-O, CH_2O-O, CH_2-S, CHMe-O, $CHCH_2NH_2$, CO-O, CHOH-O, $C(OH)_2$-O, NH-O, NH-S, NMe-O, NMe-NMe, Naryl-O, NH-NH, CH_2-CH_2, CF_2-CF_2, OCH_2O-O, O-CH_2, O-CF_2, O-NH, O-NMe, O-S, S-O, OO-O, O-SS, CC-O, C=C-O, or O_2-O then the X^2-X^4 and X^7-X^{10} are not all O or OH, and X^7-X^{10} is not one or more F or SEt when X^2-X^4 and the rest of X^7-X^{10} are O or OH;

(5) when L is CH_2O and is linked to P through O and if a X^2=P-X^7 is selected from $O=P(O)_2BH_3^-$, $O=P(O)_2NHR$, $S=P(O)_2BH_3$, $Se=P(O)_2OH$, $S=P(O)_2SH$, $S=P(O)_2NHR$, and $Se=P(O)_2SH$, at least one of X^5 or X^6 is not O; or when L is CH_2O and is linked to P through O and if a X^2=P-X^7 is selected from $O=P(O)_2BH_3^-$, $O=P(O)_2NHR$, $S=P(O)_2BH_3$, $Se=P(O)_2OH$, $S=P(O)_2SH$, $S=P(O)_2NHR$, and $Se=P(O)_2SH$, at least one of X^3-X^6, X^8-X^{10} is not O or OH;

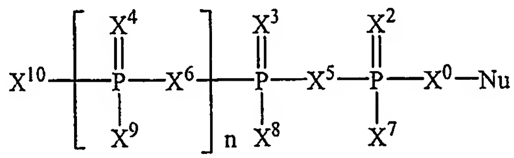
(6) when L is CH_2O or CH_2S and is linked to P through O or S respectively, one or more of X^2-X^{10} cannot be S;

(7) when L is CH_2O and is linked to P through O and one or more of X^7-X^{10} is SH, then not all X^2-X^4 is O or OH; or when L is CH_2O and is linked to P through O and one of X^7-X^{10} is SH, then X^2-X^6 and the rest of X^7-X^{10} are not all O or OH; or when L is CH_2O and is linked to P through O and two of X^7-X^{10} is SH, then X^2-X^6 and the rest of X^7-X^{10} are not all O or OH;

(8) when L is CH_2O and is linked to P through O and if the following X^6-X^5 pairs are CH_2-O, O-CH_2, NH-O, or CH_2-CH_2, then X^2-X^4 and X^7-X^{10} cannot all be O or OH, X^2-X^4 and X^7-X^{10} cannot be one or more S if the remainder of X^2-X^4 and X^7-X^{10} are O or OH, and X^2-X^4 and X^7-X^{10} cannot be one or more Me if the remainder of X^2-X^4 and X^7-X^{10} are O or OH; or when L is CH_2O and is linked to P through O and if the following X^6-X^5 pairs are CH_2-O, O-CH_2, NH-O, or CH_2-CH_2, then at least one of X^2-X^4 is not O or S; or at least of one of X^7-X^{10} is not O, S, or Me; and

(9) when L is CH_2O and linked to P through CH_2 and if A is O or CH_2 and X^{10} is alkyl, aryl, alkoxy, aryloxy, alkylamino, arylamino, or glyceroloxy, X^6 is not methylene and substituted methylene.

Further, nucleotide mimics of the invention (*i.e.*, X^7-X^{10} do not require the presence of R*), include compound of Formula **(XVI)**:



(XVI)

wherein X^0, X^5 and X^6 are selected independently from the group consisting of O, S, NH, CY_2;

wherein X^2, X^3, and X^4 are each independently O, S, or Se;

wherein X^7, X^8, X^9, and X^{10} are selected independently from the group consisting of H, F, OH, SH, NH_2, NHOH, CN, N_3, $^{-}BH_3M^+$, R, OR, SR, SeH, SeR, NHR, and NR_2;

wherein n is 0 or 1;

wherein Nu is selected from the group consisting of natural nucleosides, sugar-modified nucleosides, base-modified nucleosides, and nucleosides with both sugar and base modifications;

wherein Nu is linked to X^0 through CH_2 of the sugar moiety of Nu; and

R is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, acyl, and aralkyl each optionally containing one or more heteroatoms;

with provisos that:

(1) when L is CH_2O and is linked to P through O and if one or more of X^7-X^{10} is selected from -BH_3M^+, SH, F, SR, R, OR, NH_2, NHR, and NR_2, then X^2-X^6 and the rest of X^7-X^{10} are not all O or OH; or when L is CH_2O and is linked to P through O and if one or more of X^7- X^{10} is selected from SH, F, SR, R, OR, NH_2, NHR, and NR_2, then X^2-X^6 and the rest of X^7-X^{10} are not all O or OH;

(2) when L is CH_2O and is linked to P through O and X^7- X^{10} are OH or OR, not all X^2-X^4 are O;

(3) when L is CH_2O and is linked to P through O and if one or more of X^2-X^4 is selected from S, NH, NR, NH, then X^5-X^{10} are not all O or OH, and one of X^5-X^{10} cannot be S when the rest of X^5-X^{10} are O or OH; or when L is CH_2O and is linked to P through O and if one of X^2-X^4 is selected from S, NH, NR, NH, then X^5-X^{10} are not all O or OH, and one of X^5-X^{10} cannot be S when the rest of X^5-X^{10} are O or OH; or when L is CH_2O and is linked to P through O and if two of X^2-X^4 is selected from S, NH, NR, NH, then X^5-X^{10} are not all O or OH, and one of X^5-X^{10} cannot be S when the rest of X^5-X^{10} are O or OH

(4) when L is CH_2O and is linked to P through O and if the following X^6-X^5 pairs are CY_2-O, CH_2O-O, CH_2-S, CHMe-O, $CHCH_2NH_2$, CO-O, CHOH-O, $C(OH)_2$-O, NH-O, NH-S, NMe-O, NMe-NMe, Naryl-O, NH-NH, CH_2-CH_2, CF_2-CF_2, OCH_2O-O, O-CH_2, O-CF_2, O-NH, O-NMe, O-S, S-O, OO-O, O-SS, CC-O, C=C-O, or O_2-O then the X^2-X^4 and X^7-X^{10} are not all O or OH, and X^7-X^{10} is not one or more F or SEt when X^2-X^4 and the rest of X^7-X^{10} are O or OH;

(5) when L is CH_2O and is linked to P through O and if a X^2=P-X^7 is selected from $O=P(O)_2BH_3^-$, $O=P(O)_2NHR$, $S=P(O)_2BH_3$, $Se=P(O)_2OH$, $S=P(O)_2SH$, $S=P(O)_2NHR$, and $Se=P(O)_2SH$, at least one of X^5 or X^6 is not O; or when L is CH_2O and is linked to P through O and if a X^2=P-X^7 is selected from $O=P(O)_2BH_3^-$, $O=P(O)_2NHR$, $S=P(O)_2BH_3$, $Se=P(O)_2OH$, $S=P(O)_2SH$, $S=P(O)_2NHR$, and $Se=P(O)_2SH$, at least one of X^3-X^6, X^8-X^{10} is not O or OH;

(6) when L is CH_2O or CH_2S and is linked to P through O or S respectively, one or more of X^2-X^{10} cannot be S;

(7) when L is CH_2O and is linked to P through O and one or more of X^7-X^{10} is SH, then not all X^2-X^4 is O or OH; or when L is CH_2O and is linked to P through O and one of X^7-X^{10} is SH, then X^2-X^6 and the rest of X^7-X^{10} are not all O or OH; or when L is CH_2O and is linked to P through O and two of X^7-X^{10} is SH, then X^2-X^6 and the rest of X^7-X^{10} are not all O or OH;

(8) when L is CH_2O and is linked to P through O and if the following X^6-X^5 pairs are CH_2-O, O-CH_2, NH-O, or CH_2-CH_2, then X^2-X^4 and X^7-X^{10} cannot all be O or OH, X^2-X^4 and X^7-X^{10} cannot be one or more S if the remainder of X^2-X^4 and X^7-X^{10} are O or OH, and X^2-X^4 and X^7-X^{10} cannot be one or more Me if the remainder of X^2-X^4 and X^7-X^{10} are O or OH;

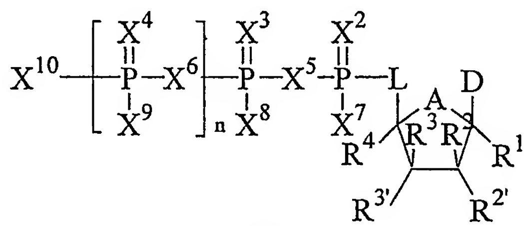
or when L is CH_2O and is linked to P through O and if the following X^6-X^5 pairs are CH_2-O, O-CH_2, NH-O, or CH_2-CH_2, then at least one of X^2-X^4 is not O or S; or at least of one of X^7-X^{10} is not O, S, or Me; and

(9) when L is CH_2O and linked to P through CH_2 and if A is O or CH_2 and X^{10} is alkyl, aryl, alkoxy, aryloxy, alkylamino, arylamino, or glyceroloxy, X^6 is not methylene and substituted methylene.

Detailed Description of the Invention

[0022] Preferred embodiments of the prodrug of Formula (I) described above include prodrugs of the following formulas:

Formula (V):



(V)

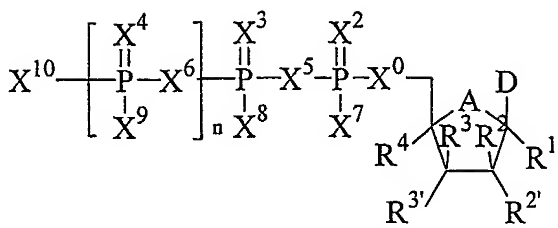
wherein L is selected from the group consisting of O, S, NH, CY_2, CY_2CY_2, and CH_2CY_2 where Y is H, F, Cl, or Br;

wherein X^2, X^3, and X^4 are O, S, Se ;

wherein X^5 and X^6 are selected independently from the group consisting of O, S, NH, NR, CY_2; and

wherein X^7, X^8, X^9, and X^{10} are selected independently from the group consisting of H, F, OH, SH, NH_2, NHOH, $^-BH_3M^+$, R, R*, OR, SR, and NHR.

Formula (VI):



(VI)

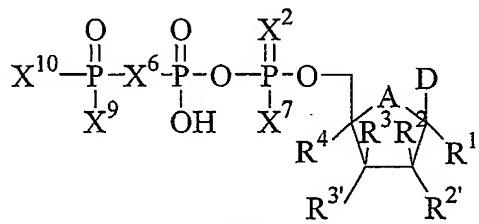
wherein X^0 is O, S, or NH;

wherein X^2, X^3, and X^4 are O or S;

wherein X^5 and X^6 are selected independently from the group consisting of O, S, NH, NR, and CY_2;

wherein X^7, X^8, X^9, and X^{10} are selected independently from the group consisting of H, F, OH, SH, NH_2, NHOH, $^-BH_3M^+$, R, R*, OR, SR, and NHR.

Formula (VII):



(VII)

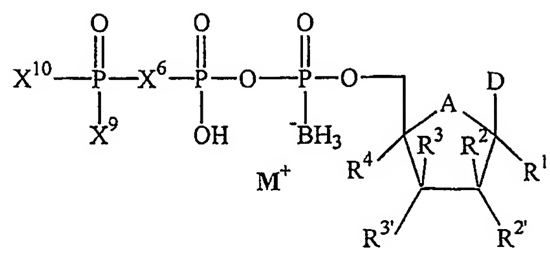
wherein X^2 is O or S;

wherein X^6 is NH or CY_2;

wherein X^7 is selected from the group consisting of H, F, SH, NH_2, NHOH, $^-BH_3M^+$, R, R*, SR, and NHR; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, NH_2, NHOH, $^-BH_3M^+$, R, R*, OR, SR, and NHR, preferably X^2 is O and X^7 is SH, NH_2, $^-BH_3M^+$, R, NHR, alkyl, aryl, alkylamino, or arylamino.

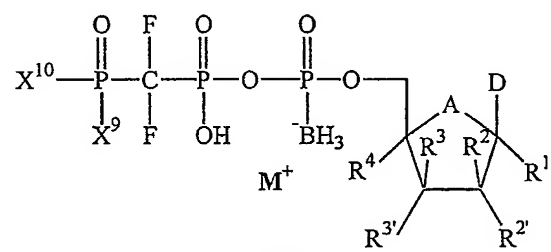
Formula **(VIII)**:



(VIII)

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, alkyl, alkoxy, aryl, and aryloxy, wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy, acylthioethoxy, acyloxymethoxy, 1,2-O-diacylglyceryloxy, and 1-O-alkyl-2-O-acylglyceryloxy; and wherein X^6 is selected from a group consisting of NH, CHF, CCl_2 and CF_2. Most preferably, at least one of X^9 or X^{10} is 1, 2,-O-dialkylglyceryloxy.

Formula **(IX)**:



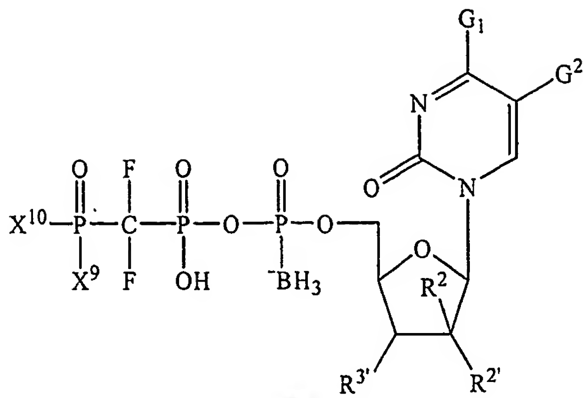
(IX)

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy, and aryloxy, or

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy, acylthioethoxy, acyloxymethoxy, 1,2-O-diacylglyceryloxy, and 1-O-alkyl-2-O-acylglyceryloxy; and

wherein X^6 is selected from a group consisting of NH, CHF, CCl_2 and CF_2.

Formula **(X)**:



(X)

wherein R^2 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

wherein $R^{2'}$ is H, F, OH, or OMe, alkyl, methoxyethoxy, or NH_2;

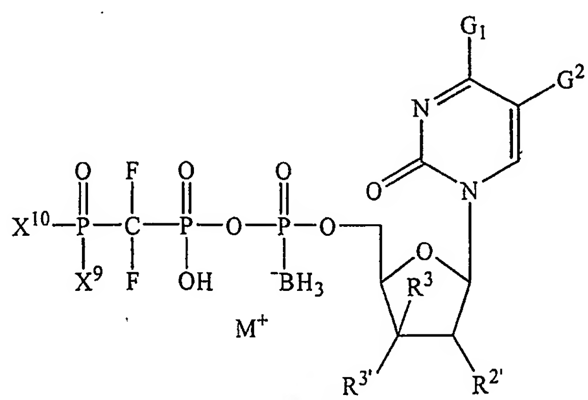
wherein $R^{3'}$ is H, F, OH, or N_3;

wherein G^1 is OH or NH_2;

wherein G^2 is H or Me;

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy, and aryloxy, or wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

Formula (XI):



(XI)

wherein $R^{2'}$ is H, F, OH, or OMe;

wherein R^3 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

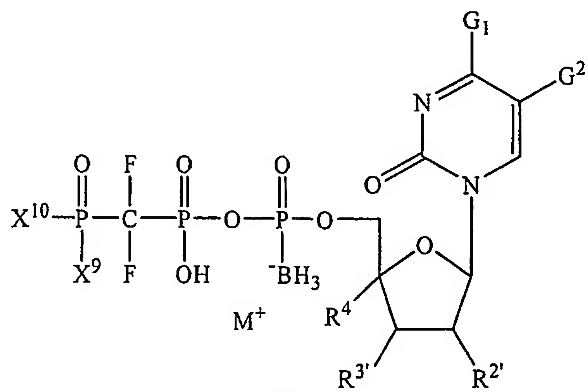
wherein $R^{3'}$ is H, F, OH, or N_3;

wherein G^1 is OH or NH_2;

wherein G^2 is H or Me; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy, and aryloxy, or wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

Formula **(XII)**:



(XII)

wherein $R^{2'}$ is H, F, OH, or OMe;

wherein $R^{3'}$ is H, F, OH, or N_3;

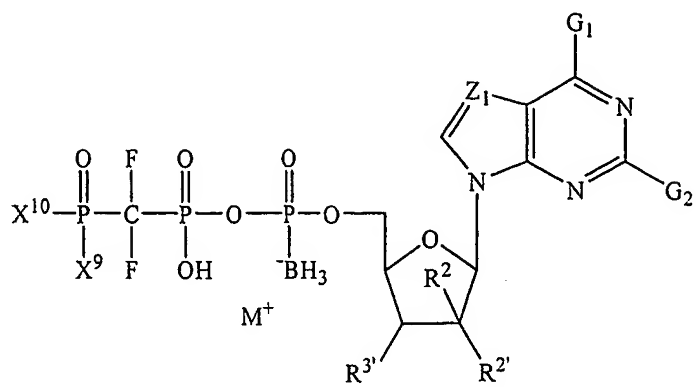
wherein R^4 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

wherein G^1 is OH or NH_2;

wherein G^2 is H or Me; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy, and aryloxy, or wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

Formula **(XIII)**:



(XIII)

wherein R^2 is methyl, ethyl, vinyl, ethynyl, hydroxymethyl or haloalkyl;

wherein $R^{2'}$ is H, F, OH, OCH_3, or methoxyethoxy;

wherein $R^{3'}$ is H, F, OH, N_3, NH_2, or CH_2OH;

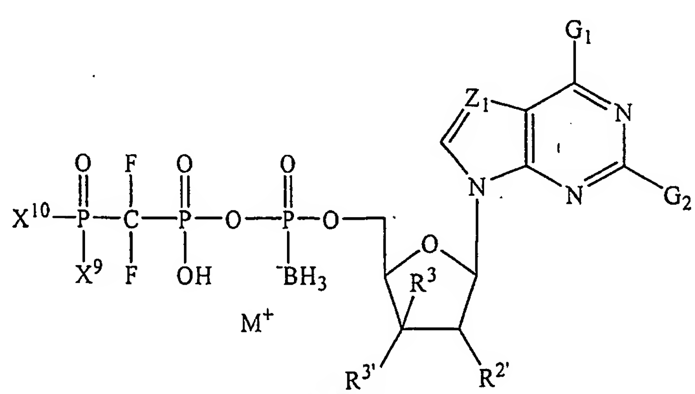
wherein G^1 is OH, NH_2, Cl, OMe, NH-cyclopropyl, SH, or S-alkyl;

wherein G^2 is H, NH_2, NHR, F, C1, Br, or I;

wherein Z^1 is N or CH; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy and aryloxy, or wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

Formula **(XIV)**:



(XIV)

wherein $R^{2'}$ is H, F, OH or OCH_3;

wherein R^3 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

wherein $R^{3'}$ is H, F, OH, or N_3;

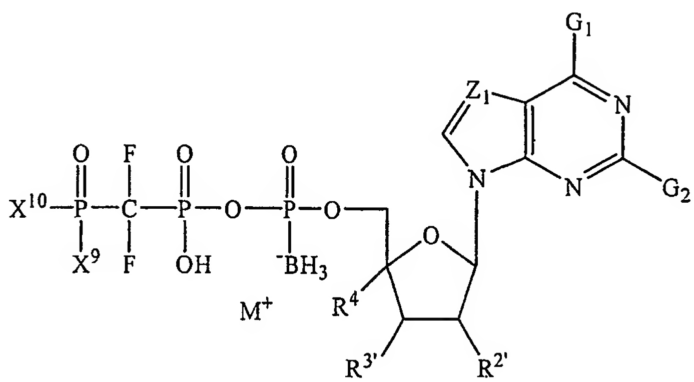
wherein G^1 is OH or NH_2;

wherein G^2 is H or NH_2;

wherein Z^1 is N or CH; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy and aryloxy, or wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

Formula **(XV)**:



(XV)

wherein $R^{2'}$ is H, F, OH or OCH_3;

wherein $R^{3'}$ is H, F, OH, or N_3;

wherein R^4 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

wherein G^1 is OH or NH_2;

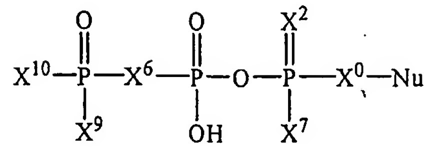
wherein G^2 is H or NH_2;

wherein Z^1 is N or CH; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy and aryloxy, or wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

[0023] Preferred embodiments of the compound of Formula **(XVI)** include prodrugs of the following formulas:

Formula **(XVII)**:



(XVII)

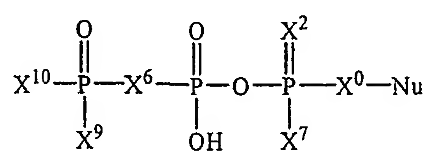
wherein X^0 and X^6 are selected independently from the group consisting of O, S, NH, CH_2, CHCl, CHBr, CHF, CCl_2, CBr_2, and CF_2

wherein X^2 is O or S;

wherein X^7 is selected from the group consisting of OH, F, SH, NH_2, NHOH, $^-BH_3M^+$, R, SR, and NHR; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, NH_2, NHOH, $^-BH_3M^+$, R, R*, OR, SR and NHR.

Formula **(XVII)**:



(XVII)

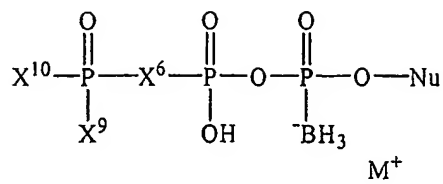
wherein X^0 and X^6 are selected independently from the group consisting of O, S, NH, CH_2, CHCl, CHBr, CHF, CCl_2, CBr_2, and CF_2

wherein X^2 is O or S;

wherein X^7 is selected from the group consisting of OH, F, SH, NH_2, NHOH $^-BH_3M^+$, R, SR, and NHR; and

wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

Formula **(XVIII)**:

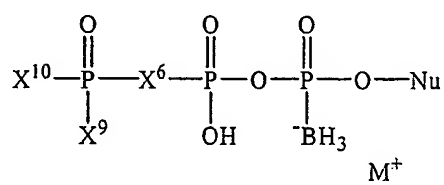


(XVIII)

wherein X^6 is selected from the group consisting of NH, CH_2, CHCl, CHBr, CHF, CCl_2, CBr_2, and CF_2; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, alkyl, alkoxy, aryl and aryloxy.

Formula **(XVIII)**:

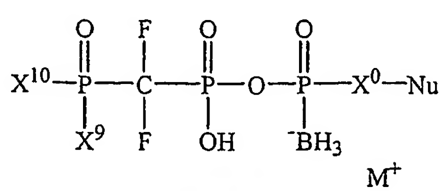


(XVIII)

wherein X^6 is selected from the group consisting of NH, CH_2, CHCl, CHBr, CHF, CCl_2, CBr_2, and CF_2; and

wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceroxy, 1,2-*O*-dialkylglyceroxy, and 1-*O*-alkyl-2-*O*-acylglyceroxy.

Formula **(XIX)**:



(XIX)

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, alkyl, alkoxy, aryl and aryloxy, or wherein X^9 and X^{10} are selected independently from the group

consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

In any of formulas **(XVI)** to **(XIX)**, Nu is preferably selected from:

adenosine,
cytidine,
guanosine,
uridine,
2'-deoxyadenosine,
2'-deoxycytidine,
2'-deoxyguanosine,
thymidine,
inosine,

9-(β–D-arabinofuranosyl)adenine,
1-(β-D-arabinofuranosyl)cytosine,
9-(β-D-arabinofuranosyl)guanine,
1-(β-D-arabinofuranosyl)uracil,
9-(β–D-arabinofuranosyl)hypoxanthine,
1-(β-D-arabinofuranosyl)thymine,

3'-azido-3'-deoxythymidine,
3'-azido-2',3'-dideoxyuridine,
3'-azido-2',3'-dideoxycytidine,
3'-azido-2',3'-dideoxyadenosine,
3'-azido-2',3'-dideoxyguanosine,
3'-azido-2',3'-dideoxyinosine,

3'-deoxythymidine,
2',3'-dideoxyuridine,
2',3'-dideoxyinosine,
2',3'-dideoxyadenosine,
2',3'-dideoxycytidine,
2',3'-dideoxyguanosine,
9-(2,3-dideoxy-1-β-D-ribofuranosyl)-2,6-diaminopurine,

3'-deoxy-2',3'-didehydrothymidine,
2',3'-didehydro-2',3'-dideoxyuridine,
2',3'-didehydro-2',3'-dideoxycytidine,
2',3'-didehydro-2',3'-dideoxyadenosine,
2',3'-didehydro-2',3'-dideoxyguanosine,
2',3'-didehydro-2',3'-dideoxyinosine,

3-deazaadenosine,

3-deazaguanosine,
3-deazainosine,
7-deazaadenosine,
7-deazaguanosine,
7-deazainosine,

6-azauridine,
6-azathymidine,
6-azacytidine,
5-azacytidine,

9-(β-D-ribofuranosyl)-6-thiopurine,
6-methylthio-9-(β-D-ribofuranosyl)purine,
2-amino-9-(β-D-ribofuranosyl)-6-thiopurine,
2-amino-6-methylthio-9-(β-D-ribofuranosyl)purine,

5-fluorocytidine,
5-iodocytidine,
5-bromocytidine,
5-chlorocytidine,
5-fluorouridine,
5-iodouridine,
5-bromouridine,
5-chlorouridine,

2'-*C*-methyladenosine,
2'-*C*-methylcytidine,
2'-*C*-methylguanosine,
2'-*C*-methylinosine,
2'-*C*-methyluridine,
2'-*C*-methylthymidine,

2'-deoxy-2'-fluoroadenosine,
2'-deoxy-2'-fluorocytidine,
2'-deoxy-2'-fluoroguanosine,
2'-deoxy-2'-fluorouridine,
2'-deoxy-2'-fluoroinosine,
2'-α-fluorothymidine,

2'-deoxy-2'-fluoroarabinoadenosine,
2'-deoxy-2'-fluoroarabinocytidine,
2'-deoxy-2'-fluoroarabinoguanosine,
2'-deoxy-2'-fluoroarabinouridine,
2'-deoxy-2'-fluoroarabinoinosine,
2'-β-fluorothymidine,

2'-*O*-methyladenosine,

2'-*O*-methylcytidine,
2'-*O*-methylguanosine,
2'-*O*-methylinosine,
2'-*O*-5-dimethyluridine,

2'-*C*-ethynylcytidine,
2'-*C*-ethynylguanosine,
2'-*C*-ethynyluridine,
2'-*C*-ethynylinosine,
2'-*C*-ethynyl-5-methyluridine,

3'-*C*-ethynyladenosine,
3'-*C*-ethynylcytidine,
3'-*C*-ethynylguanosine,
3'-*C*-ethynyluridine,
3'-*C*-ethynylinosine,
3'-*C*-ethynyl-5-methyluridine,

3'-deoxyadenosine,
3'-deoxycytidine,
3'-deoxyguanosine,
3'-deoxyuridine,
3'-deoxyinosine,

4'-*C*-ethynyladenosine,
4'-*C*-ethynylcytidine,
4'-*C*-ethynylguanosine,
4'-*C*-ethynyluridine,
4'-*C*-ethynylinosine,
4'-*C*-ethynylthymidine,

4'-*C*-methyladenosine,
4'-*C*-methylcytidine,
4'-*C*-methylguanosine,
4'-*C*-methyluridine,
4'-*C*-methylinosine,
4'-*C*-methylthymidine,

2'-*C*-methyl-7-deazaadenosine,
2'-*C*-methyl-7-deazaguanosine,
2'-*C*-methyl-3-deazaadenosine,
2'-*C*-methyl-3-deazaguanosine,
2'-*O*-methyl-7-deazaadenosine,
2'-*O*-methyl-7-deazaguanosine,
2'-*O*-methyl-3-deazaadenosine,
2'-*O*-methyl-3-deazaguanosine,
2'-*C*-methyl-6-azauridine,

2'-*C*-methyl-5-fluorouridine,
2'-*C*-methyl-5-fluorocytidine,
2'-*C*-methyl-2-chloroadenosine,

2'-deoxy-7-deazaadenosine,
2'-deoxy-3-deazaadenosine,
2'-deoxy-7-deazaguanosine,
2'-deoxy-3-deazaguanosine,
2'-deoxy-6-azauridine,
2'-deoxy-5-fluorouridine,
2'-deoxy-5-fluorocytidine,
2'-deoxy-5-iodouridine,
2'-deoxy-5-iodocytidine,
2'-deoxy-2-chloroadenosine,
2'-deoxy-2-fluoroadenosine,

3'-deoxy-7-deazaadenosine,
3'-deoxy-7-deazaguanosine,
3'-deoxy-3-deazaadenosine,
3'-deoxy-3-deazaguanosine,
3'-deoxy-6-azauridine,
3'-deoxy-5-fluorouridine,
3'-deoxy-5-iodouridine,
3'-deoxy-5-fluorocytidine,
3'-deoxy-2-chloroadenosine,

2',3'-dideoxy-7-deazaadenosine,
2',3'-dideoxy-7-deazaguanosine,
2',3'-dideoxy-3-deazaadenosine,
2',3'-dideoxy-3-deazaguanosine,
2',3'-dideoxy-6-azauridine,
2',3'-dideoxy-5-fluorouridine,
2',3'-dideoxy-5-fluorouridine,
2',3'-dideoxy-5-iodocytidine,
2',3'-dideoxy-2-chloroadenosine,

2',3'-dideoxy-β-L-cytidine,
2',3'-dideoxy-β-L-adenosine,
2',3'-dideoxy-β-L-guanosine,
3'-deoxy-β-L-thymidine,
2',3'-dideoxy-5-fluoro-β-L-cytidine,

β-L-thymidine,
2'-deoxy-β-L-cytidine,
2'-deoxy-β-L-adenosine,
2'-deoxy-β-L-guanosine,
2'-deoxy-β-L-inosine,

β-L-cytidine,
β-L-adenosine,
β-L-guanosine,
β-L-uridine,
β-L-inosine,

2',3'-didehydro-2',3'-dideoxy-β-L-cytidine,
2',3'-didehydro-3'-dideoxy-β-L-thymidine,
2',3'-didehydro-2',3'-dideoxy-β-L-adenosine,
2',3'-didehydro-2',3'-dideoxy-β-L-guanosine,
2',3'-didehydro-2',3'-dideoxy-β-L-5-fluorocytidine,

2'-deoxy-2',2'-difluorocytidine,
9-(β-D-arabinofuranosyl)-2-fluoroadenine,
2'-deoxy-2'(E)-fluoromethylenecytidine,
2'-deoxy-2'(Z)-fluoromethylenecytidine,
(-)-2',3'-dideoxy-3'-thiacytidine,
(+)-2',3'-dideoxy-3'-thiacytidine,

1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-D-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-L-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-D-ribofuranosyl-5-ethynylimidazole-4-carboxamide,
1-β-L-ribofuranosyl-5-ethynylimidazole-4-carboxamide,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine,
1-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)-5-methyluracil,
1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil,
E-5-(2-bromovinyl)-2'-deoxyuridine,
5-trifluoromethylthymidine,
1-β-D-arabinofuranosyl-5-propynyluracil,
1-(2-deoxy-2-fluoro-1-β-D-arabinofuranosyl)-5-ethyluracil,
2',3'-dideoxy-3'-fluoroguanosine,
3'-deoxy-3'-fluorothymidine,

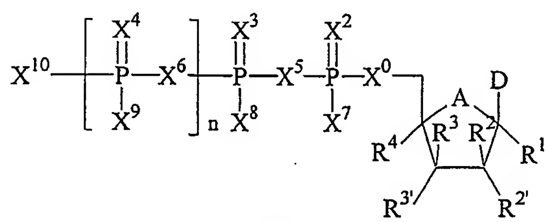
(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(1*R*,3*S*,4*R*)-9-(3-hydroxy-4-hydroxymethylcyclopent-1-yl)guanine,
(1*S*,2*R*,4*R*)-9-(1-hydroxy-2-hydroxymethylcyclopent-4-yl)guanine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)-2,6-diaminopurine,
(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)cytosine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)guanine,

(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)-5-fluorocytosine,
(1*R*,2*S*,4*S*)-9-(4-hydroxy-3-hydroxymethyl-2-methylenecyclopent-4-yl]guanine, and
(1*S*,3*R*,4*S*)-9-(3-hydroxy-4-hydroxymethyl-5-methylenecyclopent-1-yl]guanine.

[0024] The invention also relates to a pharmaceutical composition comprising a therapeutically effective amount of any of the above compounds, a pharmaceutically acceptable salt thereof, optionally in combination with one or more other active ingredients and/or with a pharmaceutically acceptable carrier. Moreover, the above any of the compounds may be used in a method for the treatment of a microbial infection or proliferative disorder comprising administering a therapeutically effective amount of any of the above compounds to a subject in need thereof.

[0025] In another embodiment of the claimed invention prefered nucleotide mimics of the compound of Formula (I) include:

Formula (VI):



(VI)

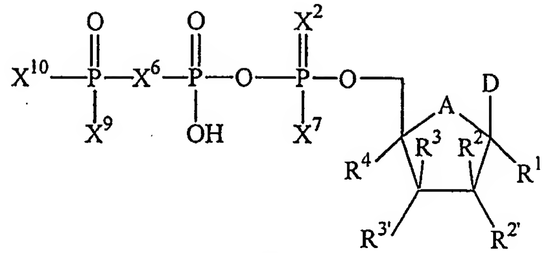
wherein X^0 is O, S, or NH;

wherein X^2, X^3, and X^4 are O or S;

wherein X^5 and X^6 are selected independently from the group consisting of O, S, NH, NR, CY_2;

wherein X^7, X^8, X^9, and X^{10} are selected independently from the group consisting of H, F, OH, SH, NH_2, NHOH, $^-BH_3M^+$, R, OR, SR, and NHR.

Formula (VII):



(VII)

wherein X^2 is O or S;

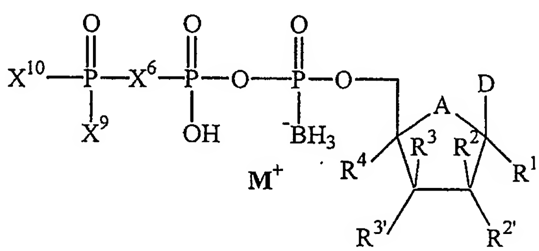
wherein X^6 is NH or CY_2;

wherein X^7 is selected from the group consisting of H, F, SH, NH_2, NHOH, $^-BH_3M^+$, R, SR, and NHR; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, NH_2, NHOH, $^-BH_3M^+$, R, OR, SR, and NHR, preferably wherein X^2 is O; and

preferably wherein X^7 is selected from the group consisting of SH, NH_2, $^-BH_3M^+$, alkyl, aryl, alkylamino, and arylamino, or wherein X^7 is selected from the group consisting of SH, NH_2, $^-BH_3M^+$, R, and NHR.

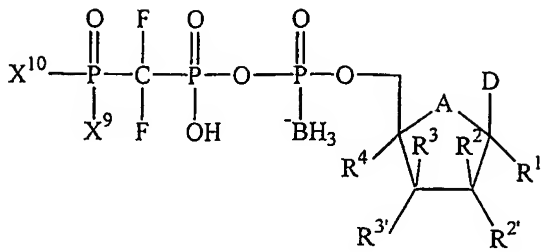
Formula (VIII):



(VIII)

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, alkyl, alkoxy, aryl, and aryloxy.

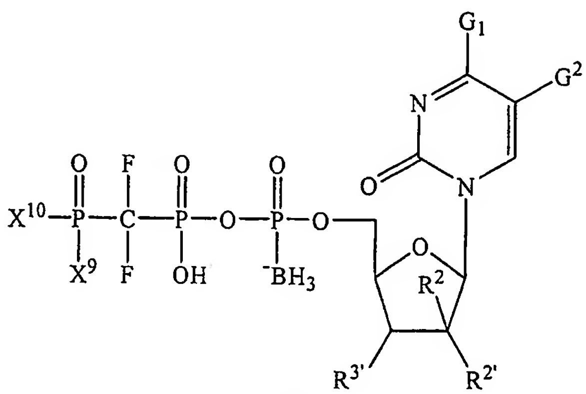
Formula **(IX)**:



(IX)

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy and aryloxy.

Formula **(X)**:



(X)

wherein R^2 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

wherein $R^{2'}$ is H, F, OH, or OMe, alkyl, methoxyethoxy, or NH_2;

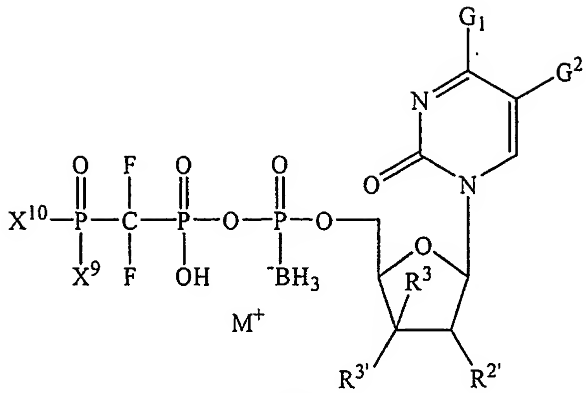
wherein $R^{3'}$ is H, F, OH, or N_3;

wherein G^1 is OH or NH_2;

wherein G^2 is H or Me; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy, and aryloxy.

Formula (XI):



(XI)

wherein $R^{2'}$ is H, F, OH, or OMe;

wherein R^3 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

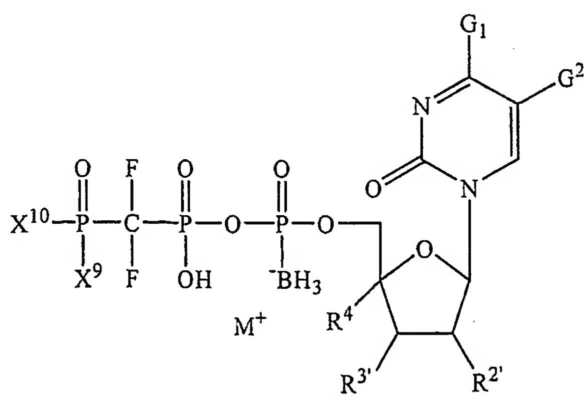
wherein $R^{3'}$ is H, F, OH, or N_3;

wherein G^1 is OH or NH_2;

wherein G^2 is H or Me; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy, and aryloxy.

Formula (XII):



(XII)

wherein $R^{2'}$ is H, F, OH, or OMe;

wherein $R^{3'}$ is H, F, OH, or N_3;

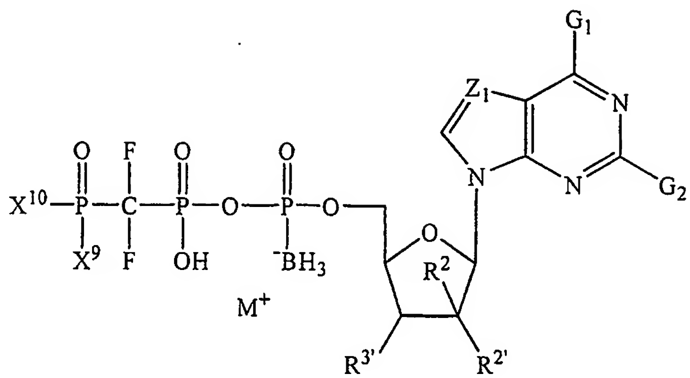
wherein R^4 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

wherein G^1 is OH or NH_2;

wherein G^2 is H or Me; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy , and aryloxy.

Formula **(XIII)**:



(XIII)

wherein R^2 is methyl, ethyl, vinyl, ethynyl, hydroxymethyl, or haloalkyl;

wherein $R^{2'}$ is H, F, OH, OCH_3, or methoxyethoxy;

wherein $R^{3'}$ is H, F, OH, N_3, NH_2, or CH_2OH;

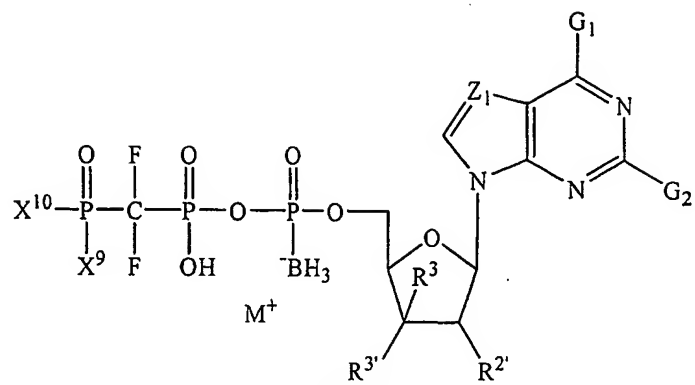
wherein G^1 is OH, NH_2, Cl, OMe, NH-cyclopropyl, SH, or S-alkyl;

wherein G^2 is H, NH_2, NHR, F, Cl, Br, or I;

wherein Z^1 is N or CH; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy and aryloxy.

Formula **(XIV)**:



(XIV)

wherein $R^{2'}$ is H, F, OH or OCH_3;

wherein R^3 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

wherein $R^{3'}$ is H, F, OH, or N_3;

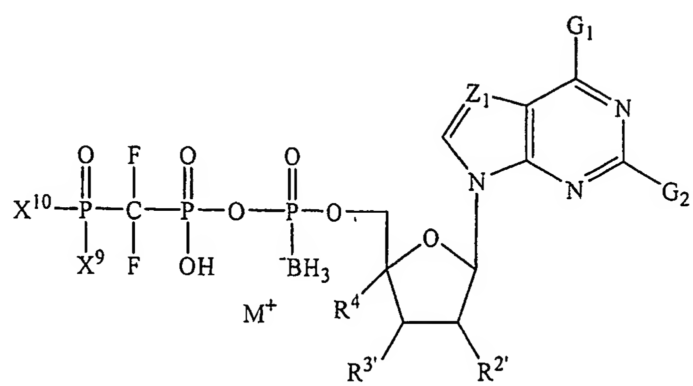
wherein G^1 is OH or NH_2;

wherein G^2 is H or NH_2;

wherein Z^1 is N or CH; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy and aryloxy.

Formula **(XV)**:



(XV)

wherein $R^{2'}$ is H, F, OH or OCH_3;

wherein $R^{3'}$ is H, F, OH, or N_3;

wherein R^4 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

wherein G^1 is OH or NH_2;

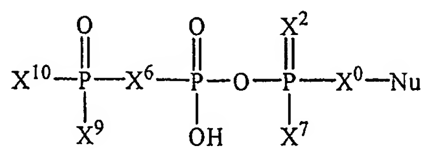
wherein G^2 is H or NH_2;

wherein Z^1 is N or CH; and

wherein X^9 and X^{10} are selected independently from the group consistir ͝of OH, alkoxy and aryloxy.

[0026] In another preferred embodiment of the claimed invention, preferred nucleotide mimics of the compound of Formula **(XVI)** include:

Formula **(XVII)**:



(XVII)

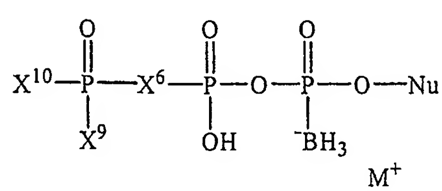
wherein X^0 and X^6 are selected independently from the group consisting of O, S, NH, CY_2;

wherein X^2 is O or S;

wherein X^7 is selected from the group consisting of OH, F, SH, NH_2, NHOH $^-BH_3M^+$, R, SR, and NHR; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, NH_2, NHOH, $^-BH_3M^+$, R, OR, SR and NHR.

Formula **(XVIII)**:

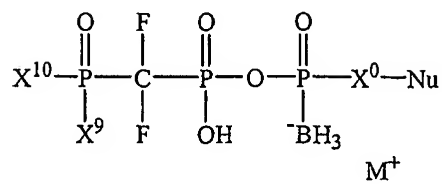


(XVIII)

wherein X^6 is NH or CY_2; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, alkyl, alkoxy, aryl and aryloxy.

Formula **(XIX)**:



(XIX)

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, alkyl, alkoxy, aryl and aryloxy.

In any formulas **(XVI)** to **(XIX)**, Nu is preferably selected from

adenosine,
cytidine,
guanosine,
uridine,
2'-deoxyadenosine,
2'-deoxycytidine,
2'-deoxyguanosine,
thymidine,
inosine,

9-(β–D-arabinofuranosyl)adenine,
1-(β-D-arabinofuranosyl)cytosine,
9-(β-D-arabinofuranosyl)guanine,
1-(β-D-arabinofuranosyl)uracil,
9-(β–D-arabinofuranosyl)hypoxanthine,
1-(β-D-arabinofuranosyl)thymine,

3'-azido-3'-deoxythymidine,
3'-azido-2',3'-dideoxyuridine,
3'-azido-2',3'-dideoxycytidine,
3'-azido-2',3'-dideoxyadenosine,
3'-azido-2',3'-dideoxyguanosine,
3'-azido-2',3'-dideoxyinosine,

3'-deoxythymidine,
2',3'-dideoxyuridine,
2',3'-dideoxyinosine,

2',3'-dideoxyadenosine,
2',3'-dideoxycytidine,
2',3'-dideoxyguanosine,
9-(2,3-dideoxy-1-β-D-ribofuranosyl)-2,6-diaminopurine,

3'-deoxy-2',3'-didehydrothymidine,
2',3'-didehydro-2',3'-dideoxyuridine,
2',3'-didehydro-2',3'-dideoxycytidine,
2',3'-didehydro-2',3'-dideoxyadenosine,
2',3'-didehydro-2',3'-dideoxyguanosine,
2',3'-didehydro-2',3'-dideoxyinosine,

3-deazaadenosine,
3-deazaguanosine,
3-deazainosine,
7-deazaadenosine,
7-deazaguanosine,
7-deazainosine,

6-azauridine,
6-azathymidine,
6-azacytidine,
5-azacytidine,

9-(β-D-ribofuranosyl)-6-thiopurine,
6-methylthio-9-(β-D-ribofuranosyl)purine,
2-amino-9-(β-D-ribofuranosyl)-6-thiopurine,
2-amino-6-methylthio-9-(β-D-ribofuranosyl)purine,

5-fluorocytidine,
5-iodocytidine,
5-bromocytidine,
5-chlorocytidine,
5-fluorouridine,
5-iodouridine,
5-bromouridine,
5-chlorouridine,

2'-*C*-methyladenosine,
2'-*C*-methylcytidine,
2'-*C*-methylguanosine,
2'-*C*-methylinosine,
2'-*C*-methyluridine,
2'-*C*-methylthymidine,

2'-deoxy-2'-fluoroadenosine,
2'-deoxy-2'-fluorocytidine,

2'-deoxy-2'-fluoroguanosine,
2'-deoxy-2'-fluorouridine,
2'-deoxy-2'-fluoroinosine,
2'-α-fluorothymidine,

2'-deoxy-2'-fluoroarabinoadenosine,
2'-deoxy-2'-fluoroarabinocytidine,
2'-deoxy-2'-fluoroarabinoguanosine,
2'-deoxy-2'-fluoroarabinouridine,
2'-deoxy-2'-fluoroarabinoinosine,
2'-β-fluorothymidine,

2'-*O*-methyladenosine,
2'-*O*-methylcytidine,
2'-*O*-methylguanosine,
2'-*O*-methylinosine,
2'-*O*-5-dimethyluridine,

2'-*C*-ethynylcytidine,
2'-*C*-ethynylguanosine,
2'-*C*-ethynyluridine,
2'-*C*-ethynylinosine,
2'-*C*-ethynyl-5-methyluridine,

3'-*C*-ethynyladenosine,
3'-*C*-ethynylcytidine,
3'-*C*-ethynylguanosine,
3'-*C*-ethynyluridine,
3'-*C*-ethynylinosine,
3'-*C*-ethynyl-5-methyluridine,

3'-deoxyadenosine,
3'-deoxycytidine,
3'-deoxyguanosine,
3'-deoxyuridine,
3'-deoxyinosine,

4'-*C*-ethynyladenosine,
4'-*C*-ethynylcytidine,
4'-*C*-ethynylguanosine,
4'-*C*-ethynyluridine,
4'-*C*-ethynylinosine,
4'-*C*-ethynylthymidine,

4'-*C*-methyladenosine,
4'-*C*-methylcytidine,
4'-*C*-methylguanosine,

4'-*C*-methyluridine,
4'-*C*-methylinosine,
4'-*C*-methylthymidine,

2'-*C*-methyl-7-deazaadenosine,
2'-*C*-methyl-7-deazaguanosine,
2'-*C*-methyl-3-deazaadenosine,
2'-*C*-methyl-3-deazaguanosine,
2'-*O*-methyl-7-deazaadenosine,
2'-*O*-methyl-7-deazaguanosine,
2'-*O*-methyl-3-deazaadenosine,
2'-*O*-methyl-3-deazaguanosine,
2'-*C*-methyl-6-azauridine,
2'-*C*-methyl-5-fluorouridine,
2'-*C*-methyl-5-fluorocytidine,
2'-*C*-methyl-2-chloroadenosine,

2'-deoxy-7-deazaadenosine,
2'-deoxy-3-deazaadenosine,
2'-deoxy-7-deazaguanosine,
2'-deoxy-3-deazaguanosine,
2'-deoxy-6-azauridine,
2'-deoxy-5-fluorouridine,
2'-deoxy-5-fluorocytidine,
2'-deoxy-5-iodouridine,
2'-deoxy-5-iodocytidine,
2'-deoxy-2-chloroadenosine,
2'-deoxy-2-fluoroadenosine,

3'-deoxy-7-deazaadenosine,
3'-deoxy-7-deazaguanosine,
3'-deoxy-3-deazaadenosine,
3'-deoxy-3-deazaguanosine,
3'-deoxy-6-azauridine,
3'-deoxy-5-fluorouridine,
3'-deoxy-5-iodouridine,
3'-deoxy-5-fluorocytidine,
3'-deoxy-2-chloroadenosine,

2',3'-dideoxy-7-deazaadenosine,
2',3'-dideoxy-7-deazaguanosine,
2',3'-dideoxy-3-deazaadenosine,
2',3'-dideoxy-3-deazaguanosine,
2',3'-dideoxy-6-azauridine,
2',3'-dideoxy-5-fluorouridine,
2',3'-dideoxy-5-fluorouridine,
2',3'-dideoxy-5-iodocytidine,

2',3'-dideoxy-2-chloroadenosine,

2',3'-dideoxy-β-L-cytidine,
2',3'-dideoxy-β-L-adenosine,
2',3'-dideoxy-β-L-guanosine,
3'-deoxy-β-L-thymidine,
2',3'-dideoxy-5-fluoro-β-L-cytidine,

β-L-thymidine,
2'-deoxy-β-L-cytidine,
2'-deoxy-β-L-adenosine,
2'-deoxy-β-L-guanosine,
2'-deoxy-β-L-inosine,

β-L-cytidine,
β-L-adenosine,
β-L-guanosine,
β-L-uridine,
β-L-inosine,

2',3'-didehydro-2',3'-dideoxy-β-L-cytidine,
2',3'-didehydro-3'-dideoxy-β-L-thymidine,
2',3'-didehydro-2',3'-dideoxy-β-L-adenosine,
2',3'-didehydro-2',3'-dideoxy-β-L-guanosine,
2',3'-didehydro-2',3'-dideoxy-β-L-5-fluorocytidine,

2'-deoxy-2',2'-difluorocytidine,
9-(β-D-arabinofuranosyl)-2-fluoroadenine,
2'-deoxy-2'(E)-fluoromethylenecytidine,
2'-deoxy-2'(Z)-fluoromethylenecytidine,
(-)-2',3'-dideoxy-3'-thiacytidine,
(+)-2',3'-dideoxy-3'-thiacytidine,

1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-D-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-L-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-D-ribofuranosyl-5-ethynylimidazole-4-carboxamide,
1-β-L-ribofuranosyl-5-ethynylimidazole-4-carboxamide,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine,
1-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)-5-methyluracil,
1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil,
E-5-(2-bromovinyl)-2'-deoxyuridine,
5-trifluoromethylthymidine,

1-β-D-arabinofuranosyl-5-propynyluracil,
1-(2-deoxy-2-fluoro-1-β-D-arabinofuranosyl)-5-ethyluracil,
2',3'-dideoxy-3'-fluoroguanosine,
3'-deoxy-3'-fluorothymidine,

(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(1*R*,3*S*,4*R*)-9-(3-hydroxy-4-hydroxymethylcyclopent-1-yl)guanine,
(1*S*,2*R*,4*R*)-9-(1-hydroxy-2-hydroxymethylcyclopent-4-yl)guanine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)-2,6-diaminopurine,
(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)cytosine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)guanine,
(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)-5-fluorocytosine,
(1*R*,2*S*,4*S*)-9-(4-hydroxy-3-hydroxymethyl-2-methylenecyclopent-4-yl]guanine, and
(1*S*,3*R*,4*S*)-9-(3-hydroxy-4-hydroxymethyl-5-methylenecyclopent-1-yl]guanine.

[0027] The invention also includes a pharmaceutical composition comprising a therapeutically effective amount of any of the above nucleotide mimic compounds, a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable prodrug thereof, optionally in combination with one or more other active ingredients and/or with a pharmaceutically acceptable carrier. Moreover any of the above mimics may be used in a method for the treatment of microbial infects or a proliferative disorder comprising administering a therapeutically effective amount of any of the above nucleotide mimic compounds to a subject in need thereof.

[0028] The definitions of certain terms and further descriptions of the above embodiments are given below.

A. Definitions

[0029] The term moiety, unless otherwise specified, refers to a portion of a molecule. Moiety may be, but is not limited to, a functional group, an acyclic chain, a phosphate mimic, an aromatic ring, a carbohydrate, a carbocyclic ring, a heterocycle or a prodrug

[0030] A "natural" nucleoside is one that occurs in nature. For the purposes of this invention the following nucleosides are defined as the natural nucleosides: adenosine, cytidine,

guanosine, uridine, 2'-deoxyadenosine, 2'-deoxycytidine, 2'-deoxyguanosine, thymidine, and inosine.

[0031] The term base, unless otherwise specified, refers to the base moiety of a nucleoside or nucleotide (a nucleobases). The base moiety is the heterocycle portion of a nucleoside or nucleotide. The base moiety of a nucleotide mimic of Formula **(I)** or **(XVI)** may be a pyrimidine derivative or analog, a purine derivative or analog, or other heterocycle. The nucleoside base may contain two or more nitrogen atoms and may contain one or more peripheral substituents. The nucleoside base is attached to the sugar moiety of the nucleotide mimic in such ways that both β-D- and β-L-nucleoside and nucleotide can be produced.

[0032] The term sugar refers to the ribofuranose of deoxyribofuranose portion of a nucleoside or nucleotide. The sugar moiety of nucleotide mimics of Formula **(I)** may contain one or more substituents at the C1-, C2-, C3-, C4, and C-5-position of the ribofuranose. Substituents may direct to either the α- or β-face of the ribofuranose. The nucleoside base that can be considered as a substituent at the C-1 position of the ribofuranose directs to the β-face of the sugar. The β-face is the side of a ribofuranose on which a purine or pyrimidine base of natural β-D-nucleosides is present. The α-face is the side of the sugar opposite to the β-face. The sugar moiety of nucleotide mimics **(XVI)** of the present invention is not limited to a ribofuranose and its derivatives, instead, it may be a carbohydrate, a carbohydrate analog, a carbocyclic ring, or other ribofuranose analog.

[0033] The term sugar-modified nucleoside refers to a nucleoside containing a modified sugar moiety.

[0034] The term base-modified nucleoside refers to a nucleoside containing a modified base moiety, relative to a base moiety found in a natural nucleoside.

[0035] The term phosphate mimic, unless otherwise specified, refers to a phosphate analog, which may be a phosphonate, phosphothioate, phosphoselenate, selenophosphate, thiophosphate, P-boranophosphate, or phosphoramidate, or a combination thereof. The term diphosphate mimic and triphosphate mimic specifically refer to a diphosphate analog and a triphosphate analog, respectively, which comprises at least one of the phosphate mimics, one of the modifications at the bridging site of diphosphate and triphosphate, or replacements of non-bridging phosphate oxygens. The modification at the bridging site, *i.e.*, in the X^5 and X^6 positions of Formulas **(II)** and **(XVI)**, includes the replacement of O by other atoms or functions

such as S, Se, O_2, NH, **NHR**, NR, CH_2, CHF, CHCl, CHBr, CF_2, CCl_2, CBr_2, CHR, $CYCO_2$, CH_2O, CHOH, $C(OH)_2$, CH_2CH_2, CC, CH=CH, $CH_2CH_2CHNH_2$, CH_2CHNH_2, CY_2OCY_2, CY_2, CY_2CY_2, and CR_2 where R is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, acyl, and aralkyl each optionally containing one or more heteroatoms. Non-bridging phosphate oxygens, *e.g.*, in X^7-X^{10} positions of Formula **(II)** can be replaced by a variety of substituents including H, F, OH, SH, NH_2, NHOH, NHOR, $NHNH_2$, NHNHR, CN, N_3, $^-BH_3M^+$, R, OR, SR, SeH, SeR, NHR, NR_2, and R* where R is as defined above, and wherein R* is a prodrug substituent. M^+ is a cation preferably a pharmaceutically acceptable cation such as Ca^{2+}, ammonium, trialkylammonium or tertaalkylammonium, *e.g.*, NH_4^+, Et_3NH^+, Bu_3NH^+, and Bu_4N^+.

[0036] The α-P, β-P, and γ-P in the diphosphate mimics and triphosphate mimics may independently adopt either *R* or *S* configurations when they become a chiral phosphorus.

[0037] The term nucleotide mimic, as used herein and unless otherwise specified, refers to a nucleoside di-phosphate mimic and/or a nucleoside tri-phosphate mimic. They may be termed as NDP mimic and NTP mimic, respectively.

[0038] The term alkyl, unless otherwise specified, refers to a saturated straight, branched, or cyclic hydrocarbon of C_1 to C_{18}. Alkyls may include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, t-butyl, cyclobutyl, n-pentyl, isopentyl, neopentyl, cyclopentyl, n-hexyl, cyclohexyl, dodecyl, tetradecyl, hexadecyl, and octadecyl.

[0039] The term alkenyl, unless otherwise specified, refers to an unsaturated hydrocarbon of C_2 to C_{18} that contains at least one carbon-carbon double bond and may be straight, branched or cyclic. Alkenyls may include, but are not limited to, olefinic, propenyl, allyl, 1-butenyl, 3-butenyl, 1-pentenyl, 4-pentenyl, 1-hexenyl, and cyclohexenyl.

[0040] The term alkynyl, unless otherwise specified, refers to an unsaturated hydrocarbon of C_2 to C_{18} that contains at least one carbon-carbon triple bond and may be straight, branched or cyclic. Alkynyls may include, but are not limited to, ethynyl, 1-propynyl, 2-propynyl, 1-butynyl, and 3-butynyl.

[0041] The term aryl, unless otherwise specified, refers to an aromatic or heteroaromatic hydrocarbon moiety. Aryls may include, but are not limited to, phenyl, biphenyl, naphthyl, pyridinyl, pyrrolyl, and imidazolyl, optionally containing one or more substituents. The

substituents may include, but are not limited to, hydroxy, amino, thio, halogen, cyano, nitro, alkoxy, alkylamino, alkylthio, hydroxycarbonyl, alkoxycarbonyl, and carbamoyl.

[0042] The term aralkyl, unless otherwise specified, refers to a moiety that contains both an aryl and an alkyl, an alkenyl, or an alkynyl. Aralkyls can be attached through either the aromatic portion or the non-aromatic position. Aralkyls may include, but are not limited to, benzyl, phenethyl, phenylpropyl, methylphenyl, ethylphenyl, propylphenyl, butylphenyl, phenylethenyl, phenylpropenyl, phenylethynyl, and phenylpropynyl.

[0043] The term acyl, unless otherwise specified, refers to alkylcarbonyl. Acyls may include, but are not limited to, formyl, acetyl, fluoroacetyl, difluoroacetyl, trifluoroacetyl, chloroacetyl, dichloroacetyl, trichloroacetyl, propionyl, benzoyl, toluoyl, butyryl, isobutyryl, pivaloyl, acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

[0044] The term heteroatom refers to oxygen, sulfur, nitrogen, selenium, borane, or halogen. When one or more heteroatoms are attached to alkyl, alkeneyl, alkynyl, acyl, aryl, or arakyl, a new functional group may be produced. For instance, when one or more heteroatoms are attached to an alkyl, substituted alkyls may be produced, including, but not limited to, fluoroalkyl, chloroalkyl, bromoalkyl, iodoalkyl, alkoxy, hydroxyalkyl, alkylamino, aminoalkyl, alkylthio, thioalkyl, azidoalkyl, cyanoalkyl, nitroalkyl, carbamoylalkyl, carboxylalkyl, acylalkyl, acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

[0045] The term halogen or halo refers to fluorine, chlorine, bromine, or iodine.

[0046] The term function refers to a substituent. Functions may include, but are not limited to, hydroxy, amino, sulfhydryl, azido, cyano, halo, nitro, hydroxycarbonyl, alkoxycarbonyl, or carboxyl, either protected or unprotected.

[0047] $R^{4'}$ of Formula **(I)** represents a combination ($-L-R^5$) of a linker moiety (L) and a phosphate moiety (R^5). L is usually either a one-atom or a two-atom linker, which may, through either side, attach to the sugar and phosphate mimic moiety. R^5 represents a diphosphate mimic or a triphosphate mimic.

[0048] R of Formula **(I)** and **(XVI)** is a univalent substituent and present on the base, sugar and phosphate mimic moieties. R is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, acyl, and aralkyl, each optionally containing one or more heteroatoms, which are as defined above.

[0049] R* is a prodrug substituent. The term prodrug, unless otherwise specified, refers to a masked (protected) form of a nucleotide mimic of Formula **(I)** or **(XVI)** when one or more of X^7-X^{10} is R*. The prodrug of the nucleotide mimic can mask the negative charges of the di-phosphate (X^7, X^8 , X^{10}) mimic or tri-phosphate (X^7-X^{10}) mimic moiety or phosphate moiety, entirely or partially, or mask a heteroatom substituted alkyl, aryl or aryalkyl (W, see below) attached to a phosphate or phosphate mimic moiety in order to enhance drug absorption and/or drug delivery into cells. The prodrug can be liberated either by cellular enzymes such as lipases, esterases, reductases, oxidases, nucleases or by chemical cleavage such as hydrolysis to release (liberate) the nucleotide mimic, preferably intracellularly. Prodrugs are often referred to as cleavable prodrugs. Prodrug substituents include, but are not limited to: proteins, antibiotics (and antibiotic fragments), D- and L-amino acids attached to a phosphate moiety or a phosphate mimic moiety via a carbon atom (phosphonates), a nitrogen atom (phosphoamidates), or an oxygen atom (phosphoesters), peptides (up to 10 amino acids) attached to a phosphate moiety or a phosphate mimic moiety via a carbon atom (phosphonates), a nitrogen atom (phosphoamidates), or an oxygen atom (phosphoesters), drug moieties attached to a phosphate moiety or a phosphate mimic moiety via a carbon atom (phosphonates), a nitrogen atom (phosphoamidates), or an oxygen atom (phosphoesters), cholesterols, folic acids, vitamins, polyamines, carbohydrates, polyethylene glycols (PEGs), cyclosaligenyls, substituted 4 to 8-membered rings, with or without heteroatom substitutions, with 1,3- phosphodiester, 1,3-phosphoamidate/phosphoester or 1,3-phosphoamidate attachments to a terminal phosphate or phosphate mimic moiety (γ or β) or connecting between an α,β or β,γ phosphate moiety or phosphate mimic moiety, acylthioethoxy, (SATE) $RCOSCH_2CH_2O$-, $RCOSCH_2CH_2O$-W-O-, $RCOSCH_2CH_2O$-W-S-, $RCOSCH_2CH_2O$-W-NH-, $RCOSCH_2CH_2O$-W-, $RCOSCH_2CH_2O$-W-CY_2-, acyloxymethoxy, $RCOOCH_2O$-, $RCOOCH_2O$-W-O-, $RCOOCH_2O$-W-S-, $RCOOCH_2O$-W-NH-, $RCOOCH_2O$-W-, $RCOOCH_2O$-W-CY_2-, alkoxycarbonyloxymethoxy, $ROCOOCH_2O$-, $ROCOOCH_2O$-W-O-, $ROCOOCH_2O$-W-S-, $ROCOOCH_2O$-W-NH-, $ROCOOCH_2O$-W-, $ROCOOCH_2O$-W-CY_2-, acylthioethyldithioethoxy (DTE) $RCOSCH_2CH_2SSCH_2CH_2O$-, $RCOSCH_2CH_2SSCH_2CH_2O$-W-, $RCOSCH_2CH_2SSCH_2CH_2O$-W-O-, $RCOSCH_2CH_2SSCH_2CH_2O$-W-S-, $RCOSCH_2CH_2SSCH_2CH_2O$-W-NH-, $RCOSCH_2CH_2SSCH_2CH_2O$-CY_2-, acyloxymethylphenylmethoxy, (PAOB) RCO_2-C_6H_4-CH_2-O-RCO_2-C_6H_4-CH_2-O-W-, RCO_2-C_6H_4-CH_2-O-W-O-, RCO_2-C_6H_4-CH_2-O-W-S-, RCO_2-C_6H_4-CH_2-O-W-NH-, RCO_2-C_6H_4-CH_2-O-W-CY_2-, 1,2-*O*-diacyl-glyceryloxy, RCOO-CH_2-

CH(OCOR)-CH_2O-, 1,2-*O*-dialkyl-glyceryloxy, RO-CH_2-CH(OR)-CH_2O-, 1,2-*S*-dialkyl-glyceryloxy, RS-CH_2-CH(SR)-CH_2O-, 1-*O*-alkyl-2-*O*-acyl-glyceryloxy, RO-CH_2-CH(OCOR)-CH_2O-, 1-*S*-alkyl-2-*O*-acyl-glyceryloxy, RS-CH_2-CH(OCOR)-CH_2O-, 1-*O*-acyl-2-*O*-alky-glyceryloxy, RCOO-CH_2-CH(OR)-CH_2O-, 1-*O*-acyl-2-*S*-alky-kglyceryloxy, RCOO-CH_2-CH(SR)-CH_2O-, or any substituent attached via a carbon, nitrogen or oxygen atom to a nucleoside di- or tri-phosphate mimic that liberates the di- or tri-phosphate mimic *in vivo.*

[0050] A combination of prodrug moieties may be attached (conjugated) to one or more X^7-X^{10} positions on a nucleoside di- or tri-phosphate mimic. W is alkyl, aryl, aralkyl as described above or a heterocycle. **[DAN: DIVALENT LANGUAGE]**

[0051] The term microbial infections refer to the infections caused by bacteria, parasite, virus or fungus. Examples of microbes that cause such infections include: *Acanthamoeba,* African Sleeping Sickness (Trypanosomiasis), amebiasis, American Trypanosomiasis (Chagas Disease), Bilharzia (Schistosomiasis), cryptosporidiosis (diarrheal disease, Cryptosporidium Parvum), Giardiasis (diarrheal disease, *Giardia lamblia*), hepatitis A, B, C, D, E, leishmaniasis (skin sores and visceral), malaria (*Plasmodium* falciparum), Salmonella enteritides infection (stomach cramps, diarrhea and fever), tuberculosis (mycobacterium tuberculosis), varicella (chicken pox), yellow fever, pneumonias, urinary tract infections (Chlamydia and Mycoplasma), meningitis & meningococcal septicemia, skin and soft tissue infections (*Staphylococcus aureus*), lower respiratory tract infections (bacterial pathogens or hepatitis C)**[Guangyi-PLEASE FILL IN].**

[0052] Common infections caused by microbes are further outlined in the following chart:

Infection	Bacteria	Fungus	Protozoa	Virus
AIDS				X
Athlete's Foot		X		
Chicken Pox				X
Common Cold				X
Diarrheal Disease	X		X	X
Flu				X
Genital Herpes				X
Malaria	X		X	
Meningitis	X			
Pneumonia	X	X		

Sinusitis	X	X		
Skin Disease	X	X	X	X
Strep Throat	X			
Tuberculosis	X			
Urinary Tract Infections	X			
Vaginal Infections	X	X		
Viral Hepatitis				X

[0053] The term pharmaceutically acceptable carrier refers to a pharmaceutical formulation which serves as a carrier to deliver negatively-charged nucleotide mimics of the present invention into cells. Liposomes, polyethylenimine, and cationic lipids are examples of those carriers.

B. Chemical synthesis

[0054] The nucleotide mimics of the present invention can be prepared using known methodologies in the practice of nucleoside and nucleotide chemistry by those who are ordinarily skilled in the art. The following descriptions are served as representative demonstrations of the preparation of the nucleotide mimics of the present invention.

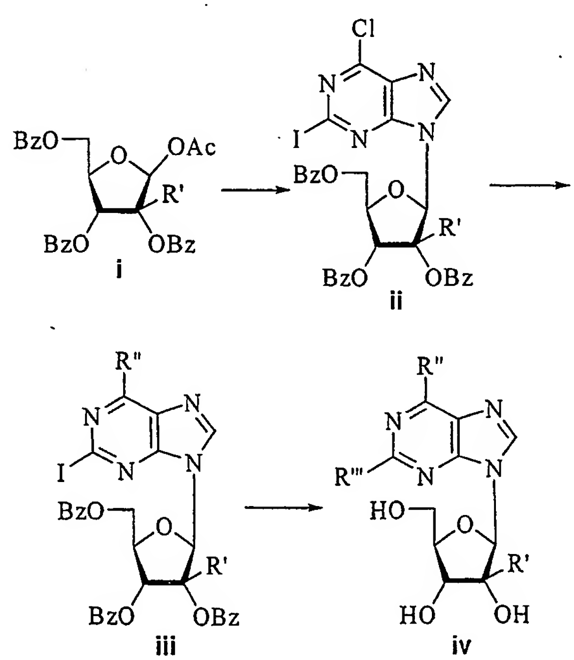
Novel nucleosides for the preparation of nucleotide mimics

[0055] The novel nucleosides that are used to prepare the nucleotide mimics of the present invention can be synthesized either according to published, known procedures or can be prepared using well-established synthetic methodologies (*Chemistry of Nucleosides and Nucleotides* Vol. 1, 2, 3, edited by Townsend, Plenum Press, 1988, 1991, 1994); *Handbook of Nucleoside Synthesis* by Vorbrüggen Ruh-Pohlenz, John Wiley & Sons, Inc., 2001; *The Organic Chemistry of Nucleic Acids* by Yoshihisa Mizuno, Elsevier, 1986). The nucleosides can be converted to their corresponding diphosphate mimics, and triphosphate mimics by established phosphorylation methodologies.

[0056] One of the general approaches for the preparation of novel nucleosides is as follow: 1. properly protected, modified sugars including 1-, 2-, 3-, 4-, 5-substituted furanose derivatives and analogs which are not commercially available need to be synthesized; 2. the modified sugars are condensed with properly substituted purine or pyrimidine derivatives and analogs to yield modified nucleosides; and 3. the resulting nucleosides can be further derivatized at nucleoside

level through reactions on the base and/or sugar moieties. Also quite often, natural nucleosides are modified directly to obtain base-modified and sugar-modified nucleosides. There are nucleoside analogs which may have to be synthesized using alternative approaches. For maximal efficiency, the nucleosides may be prepared through solution or solid-phase parallel synthesis. A representative example below (Scheme 1) can demonstrate the process. The 1-*O*-acetylribofuranose **i** is condensed with 6-chloro-2-iodopurine to yield the nucleoside **ii**, which is, at first, subject to a nucleophilic displacement of 6-chloro under a mild condition, then a nucleophilic substitution under a vigorous condition to replace iodo. The resulting compound **iii** is deprotected to yield nucleoside **iv**. For use as a starting material for the preparation of nucleotide mimics, a partial deprotection at the 5'-position may be more favorable. Thus, the 2'-, 3'- and 5'-hydroxy groups of the ribose may be protected with different protecting groups and a selective deprotection may be achieved. Here, R′, R″, and R‴ represent a substituent that may be halogen, R, NHR, NR_2, OR, or SR where R is alkyl, alkenyl, alkynyl optionally with one or more heteroatoms.

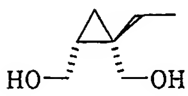
Scheme 1



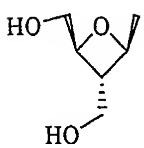
[0057] Prior publications reported a variety of ribofuranose analogs including ribofuranose derivatives, cyclopentyl derivatives, thioribofuranose derivatives, and azaribofuranose derivatives, which, with appropriate protection and substitution, can be used for the

condensations with nucleoside bases. Well-established procedures and methodologies in the literature can be used for the preparation of the modified sugars used in the present invention (Sanhvi *et al.,,Carbohydrate Modifications in Antisense Research, ACS Symposium Series,* No. 580, American Chemical Society, Washington, DC, 1994). A large number of 2- and 3-substituted ribofuranose analogs are well documented and can be readily synthesized accordingly (Hattori *et al., J. Med. Chem.* **1996**, 39, 5005-5011; Girardet *et al., J. Med. Chem.* **2000**, 43, 3704-3713)). A number of 4-, and 5-substitued sugars have also been reported and the procedures and methodologies are useful for the preparation of the modified sugars used in the invention (Gunic *et al., Bioorg. Med. Chem.* **2000**, 9, 163-170; Wang *et al., Tetrahedron Lett.* **1997**, 38, 2393-2396). Methodologies for the preparation of 4-thiosugars and 4-azasugars are also available (Rassu *et al., J. Med. Chem.* **1997**, 40, 168-180; Leydier *et al., Nucleosides Nucleotides* **1994**, 13, 2035-2050). Cyclopentyl carbocyclic sugars have been widely used to prepare carbocyclic nucleoside and the preparative procedures are also well documented (Marquez, *In Advances in Antiviral Drug Design,* De Clercq, E. Ed.; JAI press Inc. Vol. 2, 1996; pp89-146.).

[0058] In addition to ribofuranose and cyclopentyl sugars mentioned above, there are other types of novel sugars that also can be used to build novel nucleosides for the preparation of the nucleotide mimics of the present invention. Thus, the sugar moieties of the nucleotide mimics of the present invention may be selected from the group consisting of 1,2-(dihydroxymethyl)-1-ethylcycloprop-1-yl (a, b), 3,4-(dihydroxymethyl)-2-oxacyclobut-1-yl (c), 2,3-(dihydroxymethyl)cyclobut-1-yl (d), 3-hydroxymethyl-2,4-dioxacyclopent-1-yl (e), 3-hydroxymethyl-2-oxa-4-thiacyclopent-1-yl (f), 3-hydroxymethyl-2-thia-4-oxacyclopent-1-yl (g), 3-hydroxymethyl-2-methylenecyclopent-1-yl (h), 4-hydroxymethyl-2-cyclopenten-1-yl (i), and 2,3-dideoxy-2,3-didehydro-β-D-ribofuranosyl (j). The structures of these sugar moieties are shown in the chart below. In the chart the open bonding sites are the sites to where nucleoside bases are linked. The synthesis of these sugar analogs or novel nucleosides containing these sugar moieties has been reported before (Sekiyama *et al., J. Med. Chem.* **1998**, 41, 1284-1298; Norbeck *et al., Tetrahedron Lett.* **1989**, 33, 6263; Kim *et al., Tetrahedron Lett.* **1992**, 33, 6899; Jeong *et al. J. Med. Chem.* **1993**, 36, 181; Bisacchi *et al., Bioorg. Med. Chem. Lett.* **1997**, 7, 127; Huang *et al., Nucleosides Nucleotides* **1995**, 14, 195-207).


a


b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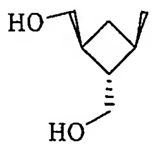
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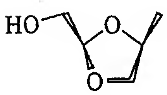
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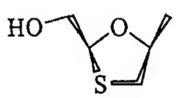
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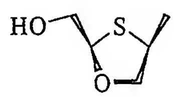
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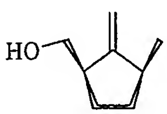
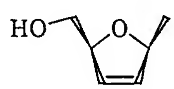

h


i


j

[0059] A variety of purine analogs, pyrimidine analogs, and other heterocycles as nucleoside bases have been well documented (*Chemistry of Nucleosides and Nucleotides* Vol 1, 2, 3, edited by Townsend, Plenum Press, 1988, 1991, 1994). The condensations of sugars with nucleoside bases to yield nucleosides are the most frequently used reactions in nucleoside chemistry. Well-established procedures and methodologies can be found in the literature (Vorbruggen *et al., Chem. Ber.* **1981**, 114, 1234-1268, 1279-1286; Wilson *et al., Synthesis*, **1995**, 1465-1479). There are several types of standard condensation reactions widely used, including: 1. trimethylsilyl triflate-catalyzed coupling reaction between 1-*O*-acetylribofuranose derivatives and silylated nucleoside bases, often used for the preparation of ribonucleosides; 2. tin chloride-catalyzed coupling reactions between 1-*O*-methyl or 1-*O*-acetylribofuranose derivatives and silylated nucleoside bases, often used to prepare 2'-deoxyribonucleosides; 3. SN2 type substitutions of 1-halosugar by nucleoside bases in the presence of a base such as sodium hydride for the preparation of both ribonucleosides and 2'-deoxyribonucleosides; and 4. less often used, but still useful, fusion reactions between sugars and nucleoside bases without solvent.

[0060] A large number of known nucleosides are prepared from the modifications of purine and pyrimidine nucleosides. The modifications can be done on the sugars and/or nucleoside bases. A simple, widely-used reaction is the nucleophilic substitution of halopurine or

halopyrimidine base by a variety of nucleophiles such as hydroxide, ammonia, hydrogen sulfide, alkoxides, amines, alkylthiol, hydrazine, hydroxyamines, azide, cyanide, and hydride. This type of reactions can be very useful for preparation of 2-substituted purine nucleoside, 6-substituted purine nucleosides, 8-susbstituted purine nucleosides, 2,6-disubstituted purine nucleosides, 2,8-disubstituted purine nucleosides, 6,8-disubstituted purine nucleosides, 2,6,8-trisubstituted purine nucleosides (Halbfinger *et al., J. Med. Chem.* **1999**, 42, 5323-5337, Lin *et al., J. Med. Chem.* **1985**, 28, 1481-1485; Bressi *et al., J. Med. Chem.* **2000**, 43, 4135-4150). These substitution reactions are readily extended to purine nucleoside analogs such as 7-deazapurine nucleosides, 7-deaza-8-azapurine nucleosides, 8-azapurine nucleosides, 3-deazapurine nucleosides, 3-deaza-8-azapurine nucleosides, and 3,8-dideazapurine nucleosides. For instance, a number of 7-deaza-7-substituted purine nucleoside have been prepared through such substitutions (Ugarkar *et al., J. Med. Chem.* **2000**, 43, 2894-2905). The same methodologies can be used for the preparation of 4-substituted pyrimidine nucleosides, 5-substituted pyrimidine nucleosides, 4,5-disubstituted pyrimidine nucleosides, 5-substituted 6-azapyrimidine nucleosides, 5-substituted 6-azapyrimidine nucleosides, and 4,5-disubstituted 6-azapyrimidine nucleosides.

[0061] The sugar moieties of synthesized nucleosides can be further modified. There are a variety of reactions which can be used to modify the sugar moiety of nucleosides. The reactions frequently used include deoxygenation, oxidation/addition, substitution, and halogenation. The deoxygenations are useful for the preparation of 2'-deoxy-, 3'-deoxy-, and 2',3'-dideoxy-nucleosides. A widely-used reagent is phenyl chlorothionoformate, which reacts with the hydroxy of nucleosides to yield a thionocarbonate. The treatment of the thionocarbonate with tributyltin hydride and AIBN yields deoxygenated nucleosides. The oxidation/addition includes the conversion of a hydroxy group to a carbonyl group, followed by a nucleophilic addition, resulting in C-alkylated nucleosides and C-substituted nucleosides. The substitution may be just a simple displacement of a hydroxyl proton by alkyl, or may be a conversion of a hydroxyl to a leaving group, followed by a nucleophilic substitution. The leaving group is usually a halogen, mesylate, tosylate, nisylate, or a triflate. A variety of nucleophiles can be used, resulting in 2-, or 3-substituted nucleosides. Halogenation can be used to prepare 1'-halo-, 2'-halo-, 3'-halo-, or 4'-halonucleosides. Chlorination and fluorination are commonly used and result in important fluoro-sugar and chloro-sugar nucleosides.

[0062] Clinically-used nucleoside drugs can be phosphorylated by cellular enzymes and maintain Watson-Crick hydrogen bonding between base pairs. However, a large number of

novel nucleosides synthesized may not be phosphorylated by a cellular enzyme and may not show *in vitro* or *in vivo* biological activities. It would be of a great value to convert some inactive nucleosides into active nucleotide mimics and to convert some moderately-active ones into potent nucleotide mimics. One approach provided in the present invention is to convert the nucleosides to the nucleotide diphosphate mimics or nucleoside triphosphate mimics, which themselves can be active chemical entities since cellular phsosphorylation is no longer necessary.

[0063] While some nucleotide mimics may be prepared from the reactions of carbohydrate-phosphate mimics with heterocycles, most of the nucleotide mimics of the present invention are prepared from nucleosides including, but not limited to, the following:

adenosine,
cytidine,
guanosine,
uridine,
2'-deoxyadenosine,
2'-deoxycytidine,
2'-deoxyguanosine,
thymidine,
inosine,

9-(β–D-arabinofuranosyl)adenine,
1-(β-D-arabinofuranosyl)cytosine,
9-(β-D-arabinofuranosyl)guanine,
1-(β-D-arabinofuranosyl)uracil,
9-(β–D-arabinofuranosyl)hypoxanthine,
1-(β-D-arabinofuranosyl)thymine,

3'-azido-3'-deoxythymidine,
3'-azido-2',3'-dideoxyuridine,
3'-azido-2',3'-dideoxycytidine,
3'-azido-2',3'-dideoxyadenosine,
3'-azido-2',3'-dideoxyguanosine,
3'-azido-2',3'-dideoxyinosine,

3'-deoxythymidine,
2',3'-dideoxyuridine,
2',3'-dideoxyinosine,
2',3'-dideoxyadenosine,
2',3'-dideoxycytidine,
2',3'-dideoxyguanosine,
9-(2,3-dideoxy-1-β-D-ribofuranosyl)-2,6-diaminopurine ,

3'-deoxy-2',3'-didehydrothymidine,
2',3'-didehydro-2',3'-dideoxyuridine,
2',3'-didehydro-2',3'-dideoxycytidine,
2',3'-didehydro-2',3'-dideoxyadenosine ,
2',3'-didehydro-2',3'-dideoxyguanosine,
2',3'-didehydro-2',3'-dideoxyinosine,

3-deazaadenosine,
3-deazaguanosine,
3-deazainosine,
7-deazaadenosine,
7-deazaguanosine,
7-deazainosine,

6-azauridine,
6-azathymidine,
6-azacytidine,
5-azacytidine,

9-(β-D-ribofuranosyl)-6-thiopurine,
6-methylthio-9-(β-D-ribofuranosyl)purine,
2-amino-9-(β-D-ribofuranosyl)-6-thiopurine,
2-amino-6-methylthio-9-(β-D-ribofuranosyl)purine,

5-fluorocytidine,
5-iodocytidine,
5-bromocytidine,
5-chlorocytidine,
5-fluorouridine,
5-iodouridine,
5-bromouridine,
5-chlorouridine,

2'-*C*-methyladenosine,
2'-*C*-methylcytidine,
2'-*C*-methylguanosine,
2'-*C*-methylinosine,
2'-*C*-methyluridine,
2'-*C*-methylthymidine,

2'-deoxy-2'-fluoroadenosine,
2'-deoxy-2'-fluorocytidine,
2'-deoxy-2'-fluoroguanosine,
2'-deoxy-2'-fluorouridine,
2'-deoxy-2'-fluoroinosine,
2'-α-fluorothymidine,

2'-deoxy-2'-fluoroarabinoadenosine,
2'-deoxy-2'-fluoroarabinocytidine,
2'-deoxy-2'-fluoroarabinoguanosine,
2'-deoxy-2'-fluoroarabinouridine,
2'-deoxy-2'-fluoroarabinoinosine,
2'-β-fluorothymidine,

2'-*O*-methyladenosine,
2'-*O*-methylcytidine,
2'-*O*-methylguanosine,
2'-*O*-methylinosine,
2'-*O*-5-dimethyluridine,

2'-*C*-ethynylcytidine,
2'-*C*-ethynylguanosine,
2'-*C*-ethynyluridine,
2'-*C*-ethynylinosine,
2'-*C*-ethynyl-5-methyluridine,

3'-*C*-ethynyladenosine,
3'-*C*-ethynylcytidine,
3'-*C*-ethynylguanosine,
3'-*C*-ethynyluridine,
3'-*C*-ethynylinosine,
3'-*C*-ethynyl-5-methyluridine,

3'-deoxyadenosine,
3'-deoxycytidine,
3'-deoxyguanosine,
3'-deoxyuridine,
3'-deoxyinosine,

4'-*C*-ethynyladenosine,
4'-*C*-ethynylcytidine,
4'-*C*-ethynylguanosine,
4'-*C*-ethynyluridine,
4'-*C*-ethynylinosine,
4'-*C*-ethynylthymidine,

4'-*C*-methyladenosine,
4'-*C*-methylcytidine,
4'-*C*-methylguanosine,
4'-*C*-methyluridine,
4'-*C*-methylinosine,
4'-*C*-methylthymidine,

2'-*C*-methyl-7-deazaadenosine,

2'-*C*-methyl-7-deazaguanosine,
2'-*C*-methyl-3-deazaadenosine,
2'-*C*-methyl-3-deazaguanosine,
2'-*O*-methyl-7-deazaadenosine,
2'-*O*-methyl-7-deazaguanosine,
2'-*O*-methyl-3-deazaadenosine,
2'-*O*-methyl-3-deazaguanosine
2'-*C*-methyl-6-azauridine,
2'-*C*-methyl-5-fluorouridine,
2'-*C*-methyl-5-fluorocytidine,
2'-*C*-methyl-2-chloroadenosine,

2'-deoxy-7-deazaadenosine,
2'-deoxy-3-deazaadenosine,
2'-deoxy-7-deazaguanosine,
2'-deoxy-3-deazaguanosine,
2'-deoxy-6-azauridine,
2'-deoxy-5-fluorouridine,
2'-deoxy-5-fluorocytidine,
2'-deoxy-5-iodouridine,
2'-deoxy-5-iodocytidine,
2'-deoxy-2-chloroadenosine,
2'-deoxy-2-fluoroadenosine,

3'-deoxy-7-deazaadenosine,
3'-deoxy-7-deazaguanosine,
3'-deoxy-3-deazaadenosine,
3'-deoxy-3-deazaguanosine,
3'-deoxy-6-azauridine,
3'-deoxy-5-fluorouridine,
3'-deoxy-5-iodouridine,
3'-deoxy-5-fluorocytidine,
3'-deoxy-2-chloroadenosine,

2',3'-dideoxy-7-deazaadenosine,
2',3'-dideoxy-7-deazaguanosine,
2',3'-dideoxy-3-deazaadenosine,
2',3'-dideoxy-3-deazaguanosine,
2',3'-dideoxy-6-azauridine,
2',3'-dideoxy-5-fluorouridine,
2',3'-dideoxy-5-fluorouridine,
2',3'-dideoxy-5-iodocytidine,
2',3'-dideoxy-2-chloroadenosine,

2',3'-dideoxy-β-L-cytidine,
2',3'-dideoxy-β-L-adenosine,
2',3'-dideoxy-β-L-guanosine,

3'-deoxy-β-L-thymidine,
2',3'-dideoxy-5-fluoro-β-L-cytidine,

β-L-thymidine,
2'-deoxy-β-L-cytidine,
2'-deoxy-β-L-adenosine,
2'-deoxy-β-L-guanosine,
2'-deoxy-β-L-inosine,

β-L-cytidine,
β-L-adenosine,
β-L-guanosine,
β-L-uridine,
β-L-inosine,

2',3'-didehydro-2',3'-dideoxy-β-L-cytidine,
2',3'-didehydro-3'-dideoxy-β-L-thymidine,
2',3'-didehydro-2',3'-dideoxy-β-L-adenosine,
2',3'-didehydro-2',3'-dideoxy-β-L-guanosine,
2',3'-didehydro-2',3'-dideoxy-β-L-5-fluorocytidine,

2'-deoxy-2',2'-difluorocytidine,
9-(β-D-arabinofuranosyl)-2-fluoroadenine,
2'-deoxy-2'(E)-fluoromethylenecytidine,
2'-deoxy-2'(Z)-fluoromethylenecytidine,
(-)-2',3'-dideoxy-3'-thiacytidine,
(+)-2',3'-dideoxy-3'-thiacytidine

1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-D-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-L-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-D-ribofuranosyl-5-ethynylimidazole-4-carboxamide,
1-β-L-ribofuranosyl-5-ethynylimidazole-4-carboxamide,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine,
1-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)-5-methyluracil,
1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil,
E-5-(2-bromovinyl)-2'-deoxyuridine,
5-trifluoromethylthymidine,
1-β-D-arabinofuranosyl-5-propynyluracil,
1-(2-deoxy-2-fluoro-1-β-D-arabinofuranosyl)-5-ethyluracil,
2',3'-dideoxy-3'-fluoroguanosine,
3'-deoxy-3'-fluorothymidine,

(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(1*R*,3*S*,4*R*)-9-(3-hydroxy-4-hydroxymethylcyclopent-1-yl)guanine,
(1*S*,2*R*,4*R*)-9-(1-hydroxy-2-hydroxymethylcyclopent-4-yl)guanine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)-2,6-diaminopurine,
(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)cytosine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)guanine,
(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)-5-fluorocytosine,
(1*R*,2*S*,4*S*)-9-(4-hydroxy-3-hydroxymethyl-2-methylenecyclopent-4-yl]guanine, or
(1*S*,3*R*,4*S*)-9-(3-hydroxy-4-hydroxymethyl-5-methylenecyclopent-1-yl]guanine.

Nucleotide mimics

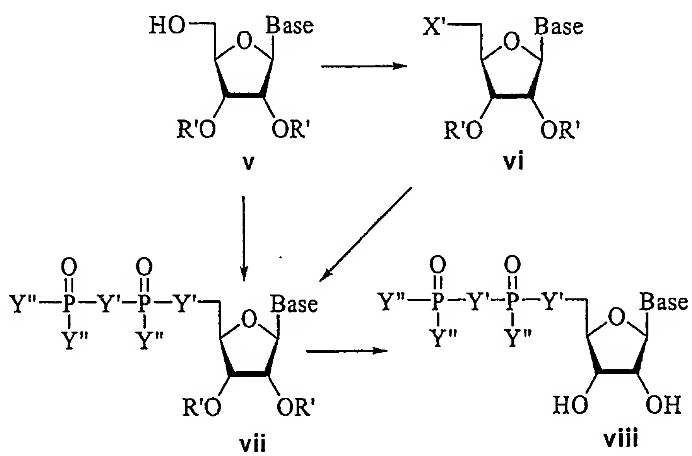
[0064] Conversion of nucleosides to nucleoside diphosphates or nucleoside triphosphates can be achieved according to published procedures. Certain nucleoside diphosphate mimics and nucleoside triphosphate mimics also have been reported. Many nucleotide mimics of the present invention can be prepared by similar approaches as published or by using well-known knowledge of organic phosphorus chemistry. Scheme 3 and 4 show the general methodologies for the preparation of nucleoside diphosphate mimics and nucleoside triphosphate mimics, respectively.

[0065] Most of the nucleotide diphosphate mimics and nucleoside triphosphate mimics of the present invention can be prepared using the methodologies shown in Scheme 2 and 3 where a protected ribonucleoside **v** is used as an example. The ribonucleoside **v** is converted to the nucleoside derivative **vi** having a leaving group such as an iodo, tosylate or triflate at the 5'-position. The reaction of **vi** with a tri- or tetraalkylammonium salt of pyrophosphate or a diphosphate mimic yields the nucleoside diphosphate mimic **vii**, which is subject to a deprotection to yield the nucleoside diphosphate mimic **viii**. Alternatively, **vii** can be prepared from the reaction of the nucleoside **v** with diphosphate mimic chloridate such as methylenediphosphonate tetrachloridate and a subsequent hydrolysis. In Scheme 3, compound **v** is converted to an activated form of nucleoside phosphate or phosphite **ix**, which is treated with a tri- or tetraalkylammonium salt of pyrophosphate or a diphosphate mimic to yield nucleoside triphosphate mimic **x**. Alternatively, **x** can be prepared from the reaction of **vii** with a tri- or teraalkylammonium salt of monophosphate or a monophosphate mimic. The deprotection of **x** affords the nucleoside triphosphate mimics **xi**. Here R' represents a protecting group such as

acetyl, benzoyl, benzyl, isopropylidene, a silyl group, trityl, or dimethoxytrityl; X' is a leaving group including halogen, and sulfonates; AP represent an activated phosphate such as monophosphate imidazolate, an active phosphine such as bis(diisopropylamino)phosphine or an activated phosphite; Y′ is O, S, NH, substituted amino, methylene, ethylene, and halomethylene; Y″ can be a variety of univalent substituents including H, OH, SH, NH_2, F, N_3, CN, and $^{-}BH_3M^{+}$, or selected from alkyl, alkoxy, alkenyl, and alkynyl optionally containing one or more heteroatoms. In addition to the general methodologies shown in Scheme 2 and 3, there are other less frequently-used methods, which are also used to prepare the nucleotide mimics of the present inventions. Some of the methods are shown in the following descriptions.

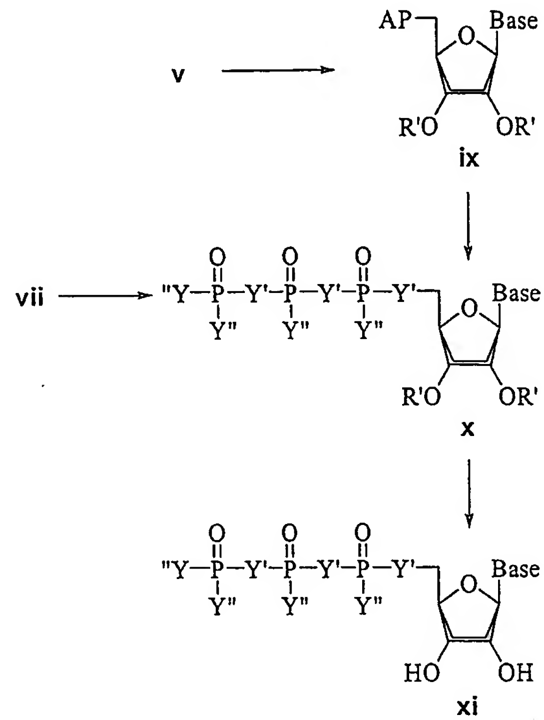
Scheme 2.

General methodologies for the preparation of nucleotide diphosphate mimics



Scheme 3.

General methodologies for the preparation of nucleotide triphosphate mimics



The purification and analysis of nucleotide mimics and their prodrugs

[0066] The compounds were purified by anion exchange (AX) chromatography using a 10 x 160 mm Mono Q column (Pharmacia). Initial conditions were typically 0 to 35 mM NaCl. Elution gradients were typically a linear gradient from 0 to 35 mM to 350 mM NaCl to 1 M NaCl in two to three column volumes at 6.5 ml/min. A constant concentration of 50 mM Tris, pH 8 was maintained throughout the purification. Fractions from anion exchange chromatography containing the target compound were collected and desalted by reversed phase (RP) chromatography using a Luna C18 250 x 21 mm column (Phenomenex) with a flow rate of 10 ml/min. Elution gradients were generally from 0-20% to 95% methanol in 20-60 min at a constant concentration of triethylammonium acetate (50 mM). Compounds that did not require AX-HPLC purification were purified by Reverse-phase HPLC only. However, most nucleotide mimics in the example section, unless specified, were purified using both ion-exchange HPLC and reverse-phase HPLC as indicated simply by HPLC.

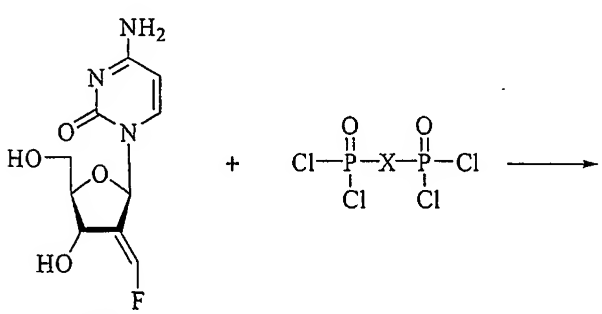
[0067] Mass Spectra and purity of the nucleotide mimics and their prodrugs were determined using on-line HPLC mass spectrometry on a ThermoFinnigan (San Jose, CA) Deca

XP plus. A Phenomenex Luna (C18(2) or c5), 75 x 2 mm, 3-um particle size was used for RP HPLC. A 0 to 50% linear gradient (15 min) of AcCN in 10 mM NNDMHA, pH 7 was performed in series with mass spectra detection in the negative ionization mode. Nitrogen gas and a pneumatic nebulizer were used to generate the electrospray. The mass range of 150 –1500 was typically sampled.

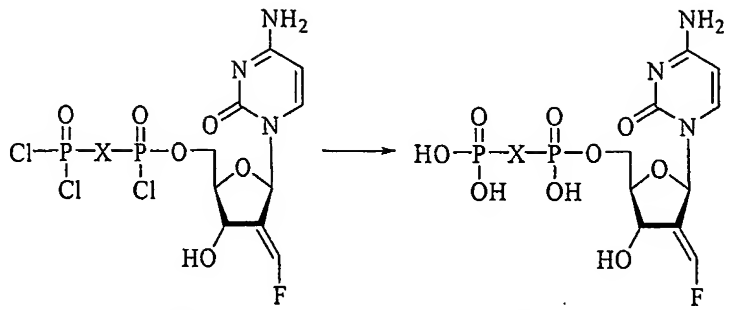
The preparation of nucleoside 5'-diphosphate mimics

[0068] In many cases, 3'-azido-3'-deoxythymidine serves as an example to illustrate various approaches and reactions. The approaches and methodologies shown below are general and can be applied to the preparation of other nucleotide mimics of the present invention although in some cases nucleosides may need appropriate protection. The detailed procedures are given in the section of Examples.

[0069] The preparation of nucleoside 5'-diphosphate mimics can be readily achieved through the reactions of nucleosides with modified diphosphotetrachloridates $Cl_2P(O)$-X-$P(O)Cl_2$ (X = NH, CH_2, CF_2, CCl_2, CHF, etc). For example, 2'-deoxy-2'-fluoromethylenecytidine was reacted with trichloro[(dichlorophosphoryl)imido]-phosphorane and methylenediphosphotetrachloridate to give, after hydrolysis, 2'-deoxy-2'-fluoromethylenecytidine 5'-imidodiphosphate (**3**) and 2'-deoxy-2'-fluoromethylenecytidine 5'-methylenediphosphonate (**4**), respectively. Similarly, the nucleoside ethylenediphosphonate **80** was prepared. A number of other nucleoside 5'-diphosphate mimics were prepared through similar reactions, including 2'-deoxy-2',2'-difluorocytidine 5'-diphosphate mimics (**5-7, 81**) and 3'-azido-3'-deoxythymidine 5'-imidodiphosphate (**8**).

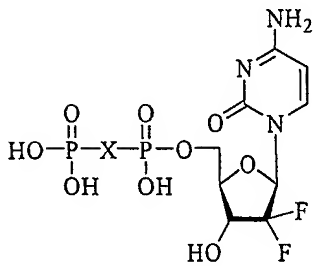


(1)

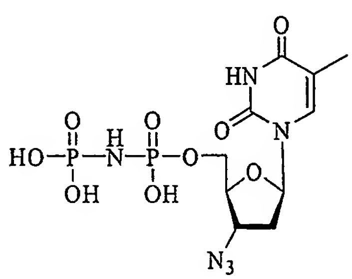


(2)

3 X = NH
4 X = CH_2
80 X = CH_2CH_2

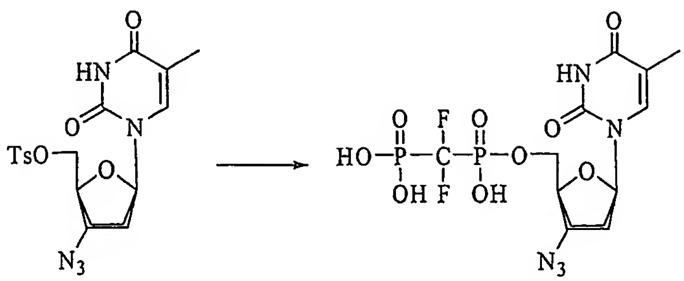


5 X = CH_2
6 X = NH
7 X = CF_2
81 X = CH_2CH_2



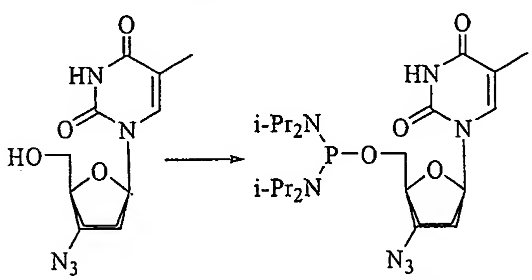
(8)

[0070] An alternative approach is to convert the 5'-hydroxy of a nucleoside to a leaving group, which is reacted with the tetrabutylammonium salt of diphosphate mimics to give nucleoside diphosphate mimics. For example, 3'-azido-3'-deoxythymidine was converted to the 5'-tosylate **9**, which was treated with the tetrabutylammonium salt of difluoromethylenediphosphonate to give 3'-azido-3'-deoxythymidine 5'-(difluoromethylene)diphosphonate (**10**).



(9) (10)

[0071] 3'-Azido-3'-deoxythymidine 5'-α-*P*-borano-β-*P*-methyldiphosphate (**13**) was prepared through the activated phoshoramidite **12**. The reaction of **12** with excess methylphosphonate monotributylammonium salt, followed by treatment with borane diisopropylethyl amine complex and subsequent hydrolysis to give the diphosphate mimic **13**. Through this approach a variety of nucleoside 5'-β-*P*-substituted diphosphates can be prepared.



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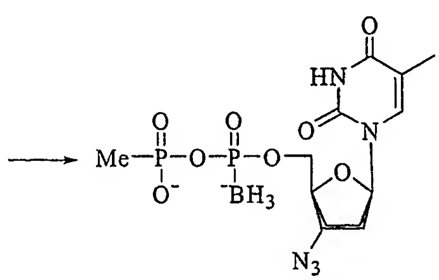
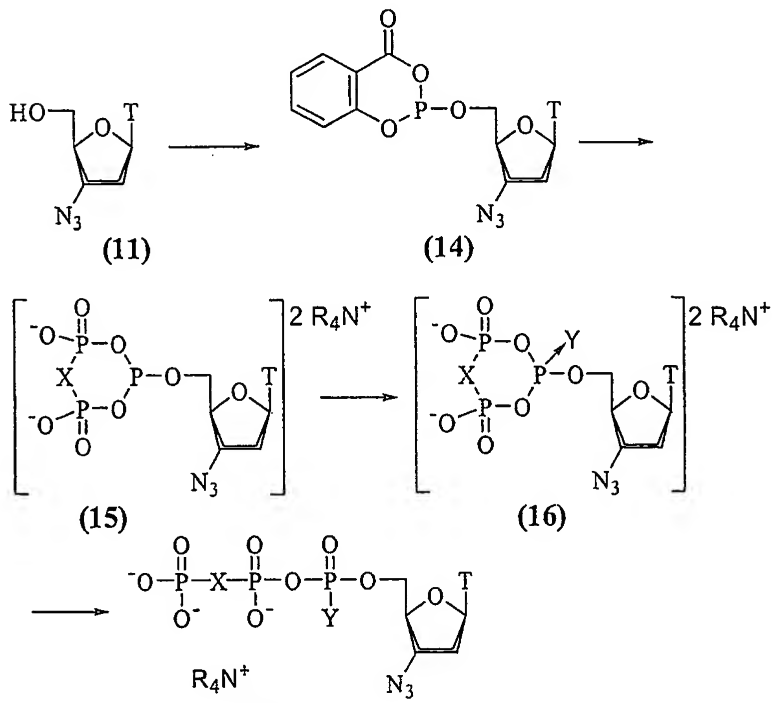


(13)

<u>The preparation of nucleoside triphosphate mimics</u>

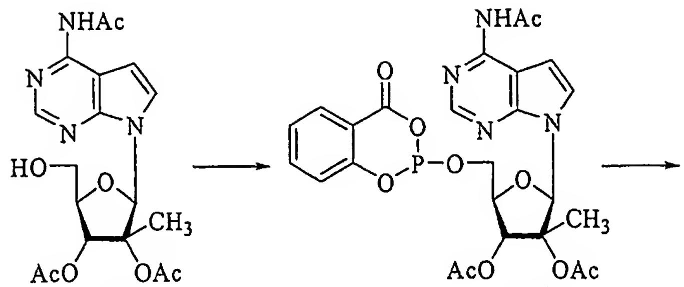
[0072] Since triphosphate mimics are more diverse than diphosphate mimics, there are more synthetic approaches for the preparation of nucleoside triphosphate mimics. One approach includes the preparation of an activated phosphate or phosphite linked to the 5'-position of a nucleoside and a subsequent condensation with diphosphate mimics. Many nucleoside triphosphate mimics of the present invention were prepared through this approach. For example, the reaction of 3'-azido-3'-deoxythymidine with 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-

one gives the intermediate **14**, which is condensed with diphosphate mimics to form a cyclic triphosphate intermediate **15**. The treatments of the cyclic triphosphate intermediate **15** with different reagents yield, after hydrolysis, a variety of nucleoside triphosphate mimics **17**. The scheme below demonstrates the reaction path. Here, X is O, S, NH, methylene, or halomethylene; Y is O, S, N_3, alkoxy, aryloxy, alkylamino, arylamino, or $^-BH_3M^+$; R_4N^+ is an ammonium, trialkylammonium, or tetraalkylammonium cation such as NH_4^+, Et_3NH^+, Bu_3NH^+, and Bu_4N^+.



(11) (14) (15) (16) (17)

[0073] When the cyclic intermediate **15** was treated with BH_3-amine complex or BH_3-sulfide complex, followed by hydrolysis with water, a number of nucleoside 5'-α-*P*-boranotriphosphate mimics were prepared. This methodology is a general method and can be applied to a variety of nucleosides, however, an appropriate protection is necessary for nucleosides bearing reactive groups. A representative example is shown below. 7-Deaza-2'-*C*-methyladenosine (**18**) was converted to 7-deaza-2'-*C*-methyl-2',3'-*O*-6-triacetyladenosine (**20**), which was subject to the reactions shown above to give the triacetyl nucleoside triphosphate mimic **20**. A deprotection with aqueous ammonia or methanolic ammonia yielded the nucleoside triphosphate mimic **22**.



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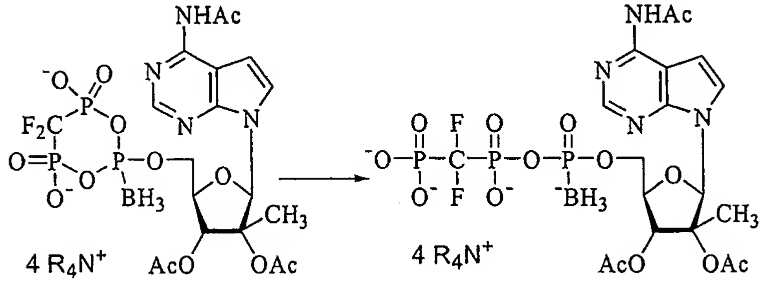

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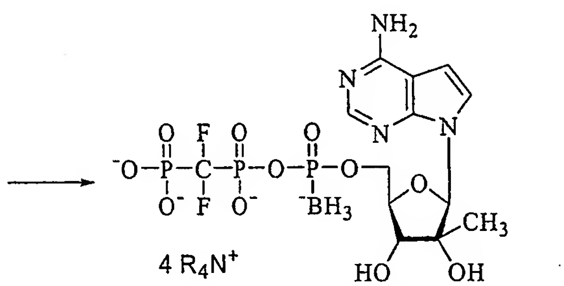

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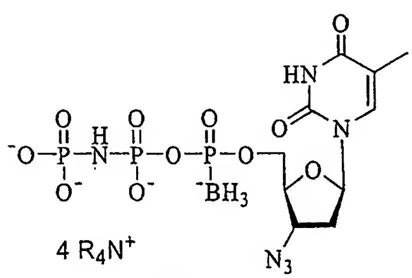
[0074] Other representative nucleoside 5'-α-*P*-boranotriphosphate mimics **23-28** containing different β,γ-bridging modifications were synthesized through the same approach and are shown below.



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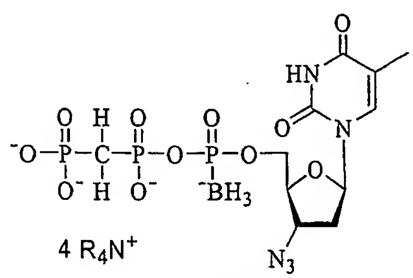

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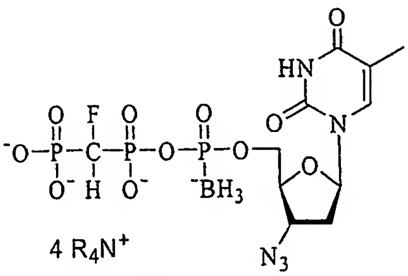

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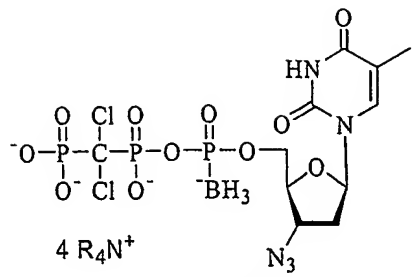

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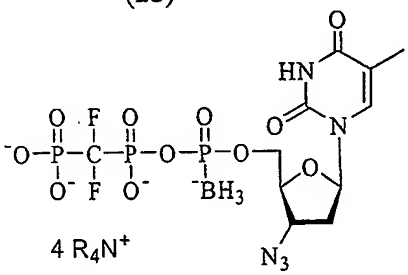

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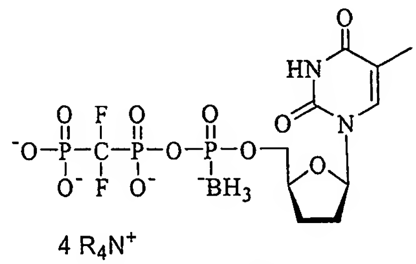
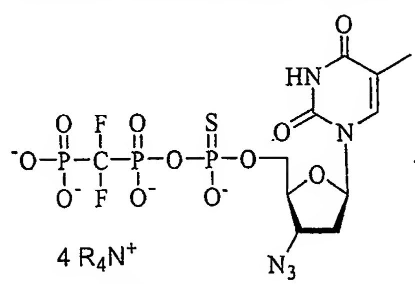


(28)

[0075] Similarly, the treatment of **15** with elemental sulfur and subsequent reaction with water gave the α-*P*-thiotriphosphate mimics **29**. Compound **30** was also prepared through the same method. Compound **31** was obtained from the treatment of a cyclic triphosphate equivalent to **15** with sulfur and subsequent reaction with lithium sulfide. Similarly, compound **32** was prepared from the treatment of the cyclic triphosphate with borane-diisopropylethylamine complex and subsequent reaction with lithium sulfide.



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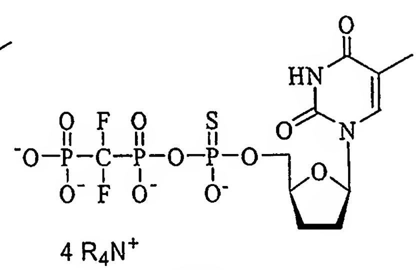

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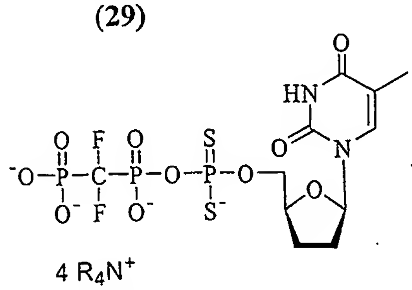

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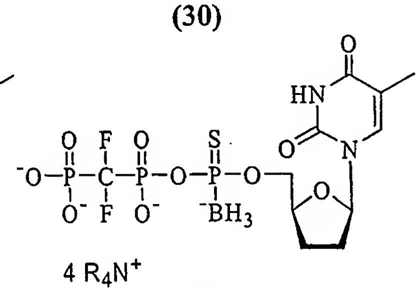
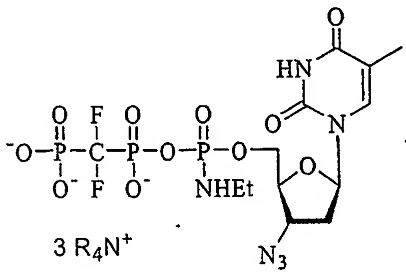


(32)

[0076] Alternatively, the treatment of the cyclic triphosphate intermediate **15** with iodine followed by reaction with a nucleophile can yield a variety of nucleoside 5'-α-*P*-substituted triphosphate mimics. For example, the reaction of **15** with iodine, followed by treatment with

ethylamine and aniline yielded the α-*P*-aminotriphosphate mimics **33** and **34**, respectively. When potassium fluoride, sodium azide, sodium phenoxide, and sodium methoxide were chosen as the nucleophile, compounds **35-38** were prepared, respectively. Compound **39** was also formed when the nucleophile was hydroxyamine.



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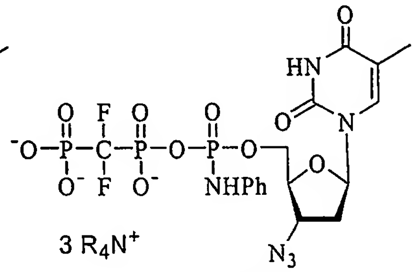

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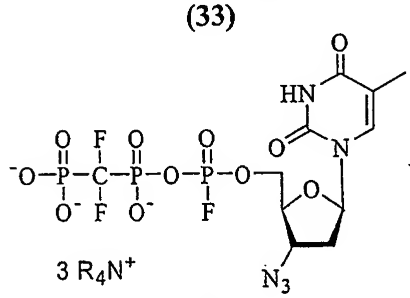

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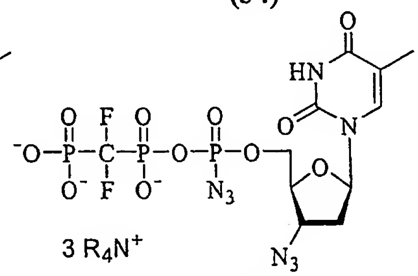

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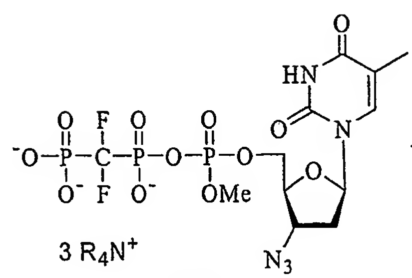

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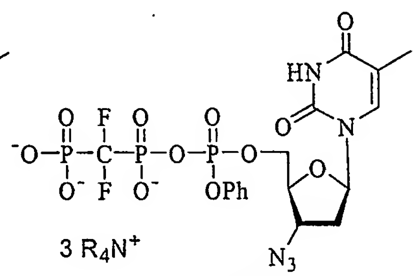

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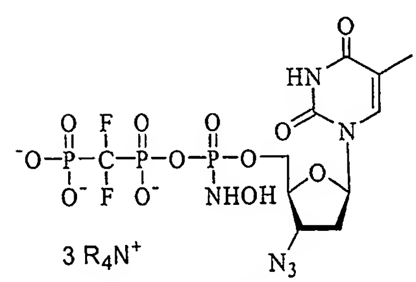
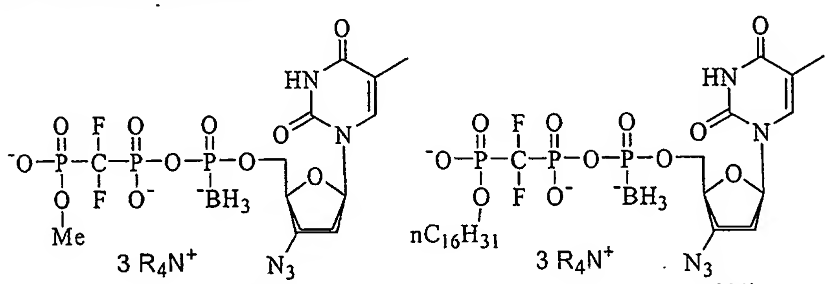


(39)

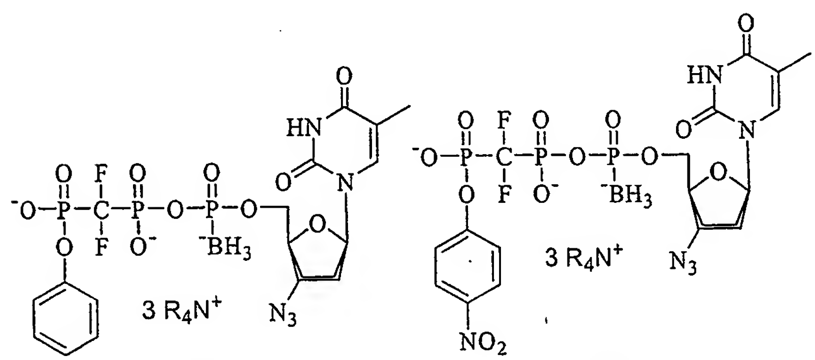
[0077] Nucleoside 5'-γ-*P*-substituted triphosphate mimics can be prepared through a few different approaches. One approach is the alkylation of nucleoside triphosphate mimics with a reagent bearing a leaving group. The alkylating reagent may be a halide such as a bromide and iodide or a sulfonate such as a tosylate, nisylate, and triflate. This type of reaction can be used to

introduce a small aliphatic hydrocarbon or a large moiety such as lipids and other phosphate-masking moieties. For example, the reaction of the tetrabutylammonium salt of the nucleotide mimic **27** with methyl triflate yielded the γ-*P*-methyltriphosphate mimic **40**. Similarly, the γ-*P*-palmityltriphosphate mimic **41** was prepared from the reaction of **27** with palmityl *p*-nitrobenzenesulfonate.



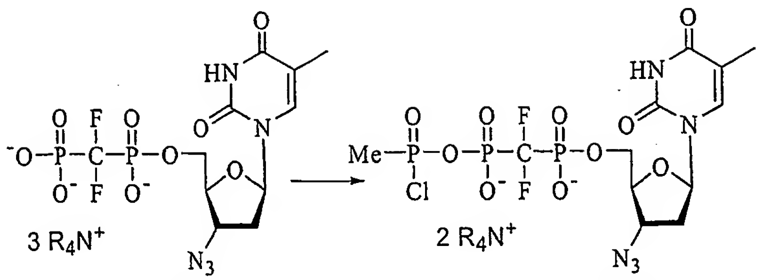
(40) **(41)**

[0078] Compounds **42** and **43** were prepared, respectively, through the treatment of the nucleoside triphosphate mimic **27** with DCC and subsequent reaction with phenol and *p*-nitrophenol. This reaction represents a general approach for the attachment of aryloxy substituents at the γ-P position of nucleoside triphosphate mimics.



(42) **(43)**

[0079] Another approach for the preparation of the nucleoside 5'-γ-*P*-substituted triphosphate mimics is the reaction of nucleoside diphosphate mimics with substituted phosphonodichloridate as shown below. For example, the treatment of 3'-azido-3'-deoxythymidine 5'-(difluoromethylene)diphosphonate (**10**) with methylphosphonodichloridate yields, after hydrolysis, the triphosphate mimics **44**. Similarly, the compound **45** containing γ-*P*-phenyl-α,β-(difuoromethylene)triphosphate was prepared.



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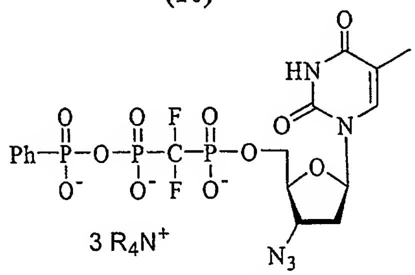

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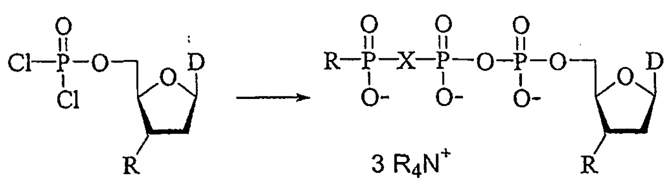
[0080] An additional approach for the preparation of nucleoside 5'-γ-*P*-substituted triphosphate mimics **47** is the reaction of nucleoside 5'-phosphodichloridate **46** or 5'-phophonodichloridate with substituted pyrophosphate or substituted diphosphate mimics. Also, the reaction of the nucleoside cyclic phosphate **14** with substituted pyrophosphate or substituted diphosphate mimics, followed by treatment with oxidizing reagents such as sulfur, iodine, and borane and then with nucleophiles, can yield a variety of nucleoside 5'-γ-*P*-(and/or α-*P*)-substituted triphosphate mimics **48**. Through this approach substituents such as γ-*P*-alkyl, γ-*P*-alkylamino, γ-*P*-alkylthio, γ-*P*-alkoxy, γ-*P*-aryl, γ-*P*-aryloxy, and γ-*P*-arylamino can be introduced.



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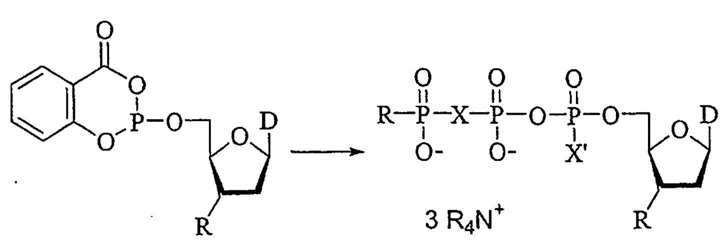
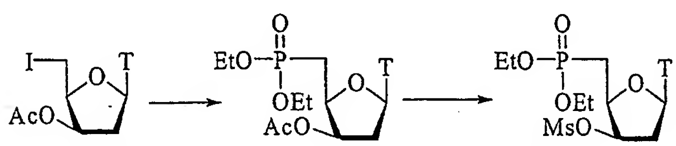


(14) (48)

[0081] The present invention also provides approaches for the preparation of nucleoside triphosphate mimics in which the modifications are present at the 5'-position of the nucleotide

mimics. The 5'-oxygen of nucleotide mimics can be replaced with other heteroatoms, methylene, halomethylene or dihalomethylene. Alternatively, the 5'-oxygen can be eliminated. Two examples shown below are used to illustrate the preparation of these compounds. The first scheme shows the synthesis of 3'-azido-3'-deoxythymidine 5'-deoxy-β,γ-(difluoromethylene)triphosphate. 1-(2-Deoxyxylofuranosyl)thymine was converted to a 5'-iodo derivative, which was subjected to acetylation. The resulting intermediate **49** was converted to the 5'-deoxy-5'-phosphonate ester **50**. The 3'-acetyl was removed and the resulting intermediate was converted to the mesylate **51**. The treatment of **51** with sodium azide at elevated temperature gave the 3'-azido derivative **52**. After removal of the ethyl groups, the tributylammonium salt of the phosphonate **53** was treated with carbonyldiimidazole, and the resulting intermediate **54** was condensed with the tributylammonium salt of difluoromethylenediphosphonate to yield 3'-azido-3',5'-dideoxythymidine 5'-β,γ-(difluoromethylene)triphosphate **55**. Compounds **56** and **57** were prepared through similar procedures.



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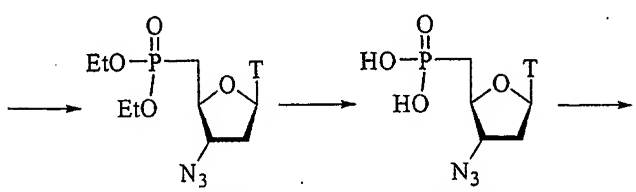

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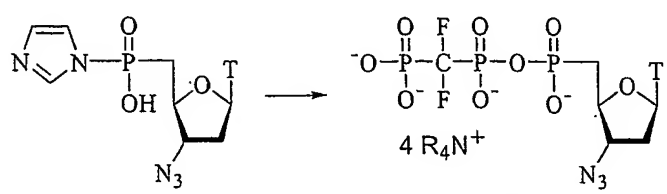

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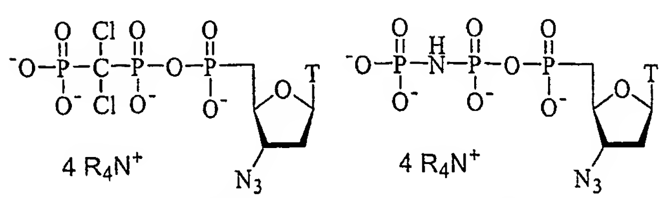
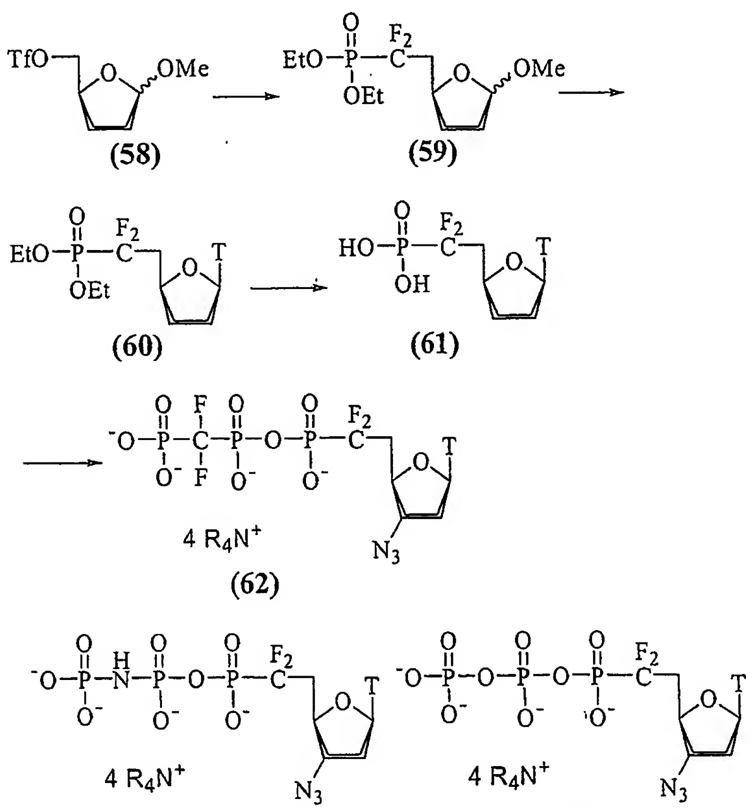


(56) (57)

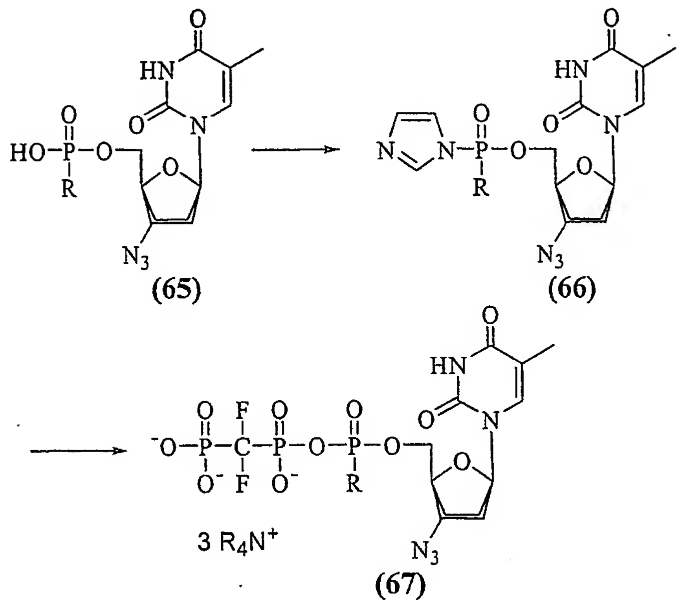
[0082] The scheme below shows the synthesis of 3',5'-dideoxy-5'-difluoromethylenethymidine 5'-β,γ-(difluoromethylene)triphosphate (**62**). 2,3-Dideoxy-1-*O*-methylribofuranose was converted to the 5-*O*-triflate **58**, which was treated with LDA and then reacted with diethyl *P*-difluoromethylphosphonate to give the 5-deoxy-5-difluoromethylene derivative **59**. The condensation of **59** with a silylated thymine in the presence of tin chloride gave the nucleoside 5'-phosphonate ester **60**. After removal of the ethyl groups with TMSBr, the triethylammonium salt of the nucleoside 5'-phosphonate **61** was treated with carbonyldiimidazole, and then condensed with the tributylammonium salts of difluoromethylenediphosphonate to give 3',5'-dideoxy-5'-difluoromethylenethymidine 5'-β,γ-(difluoromethylene)triphosphate (**62**). Compounds **63** and **64** were prepared through similar procedures.



(58) (59) (60) (61) (62) (63) (64)

[0083] Another general approach for the preparation of α-*P*-substituted nucleoside triphosphates can be used for the preparation of the nucleotide mimics of the present invention, involving the reaction of an activated nucleoside monophosphonate with pyrophosphate or diphosphate mimics. The activation is usually achieved by attaching an imidazole or a

morpholino group to the phosphate, as shown by the example given below. 3'-Azido-3'-deoxythymidine is converted to its 5'-phosphonate **65** (R = alkyl or aryl), which can be activated by treatment with carbonyldiimidazole. The resulting intermediate **66** is condensed with the tetrabutylammonium salt of difluoromethylenediphosphonate to give 3'-azido-3'-deoxythymidine 5'-α–*P*-substituted β,γ-(difluoromethylene)triphosphate (**67**).

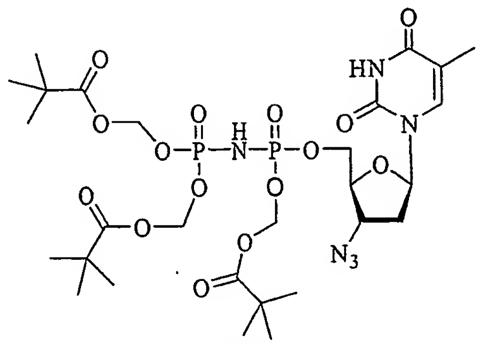


Nucleotide mimic prodrugs

[0084] The prodrug approach is one of the efficient methods to deliver polar, negatively-charged nucleotide mimics into cells. A number of prodrug approaches for nucleoside 5'-monophosphates have been developed and potentially can be applied to the nucleotide mimics of the present invention. The nucleotide mimic prodrugs may include, but are not limited to, alkyl phosphate esters, aryl phosphate ester, acylthioethyl phosphate esters, acyloxymethyl phosphate esters, 1,2-*O*-diacylglyceryl phosphate esters, 1,2-*O*-dialkylglyceryl phosphate esters, and phosphoramidate esters. These masking groups were also successfully attached to the nucleoside mimics of the present invention. The resulting compounds can serve as the prodrugs of the nucleotide mimics.

[0085] One of the straightforward methods for the preparation of nucleotide mimic prodrugs is the reaction of the nucleotide mimics with a masking reagent bearing a leaving group. For example, the nucleoside 5'-imidodiphosphate **8** is treated with tributylstannyl methoxide to convert the diphosphate **8** to a stannic salt, which was reacted with pivaloxymethyl iodide to

give compound **68**. The pivaloxymethyl group (POM) can be cleaved by a variety of cellular esterases. In cells compound **68** can be converted to compound **8**. Compound **69** was prepared from the reaction of the corresponding triphosphate **27** with pivaloxymethyl iodide through a similar procedure. Compound **70** was also prepared through this type of reaction, but the reagent is *S*-pivaloyl-2-thioethyl (SATE) *p*-nitrobenzenesulfonate. *S*-Pivaloyl-2-thioethyl and pivaloxymethyl as the making groups of phosphate have been intensively studied and usually abbreviated as SATE and POM, respectively. Like POM, SATE can also be readily cleaved by cellular esterases.



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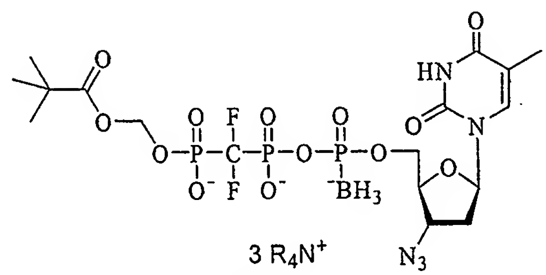

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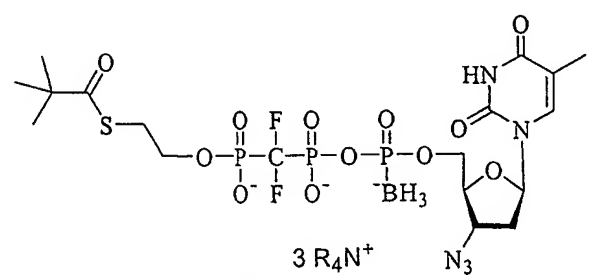

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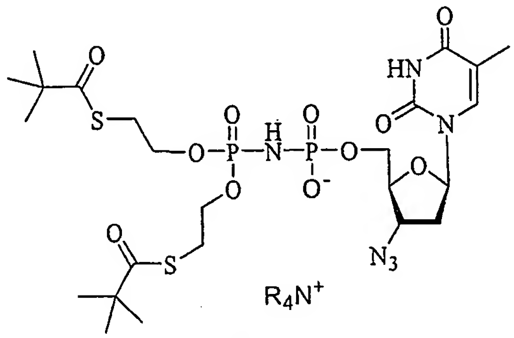
[0086] Another approach for the preparation of the prodrugs from nucleotide mimics is the condensation of nucleotide mimics with a masking group bearing a hydroxyl group. For example, compound **8** was treated with 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole and then

reacted with *S*-pivaloxy-2-thioethanol to give compounds **71** and **72**, which were separated by chromatography.



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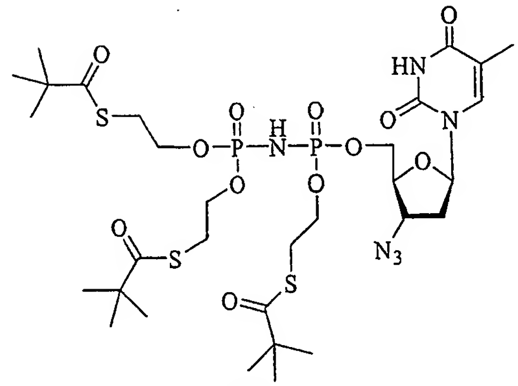
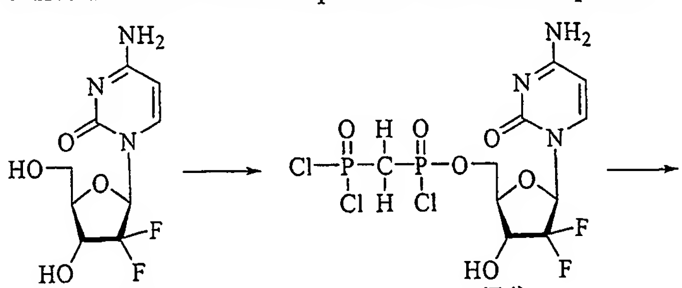
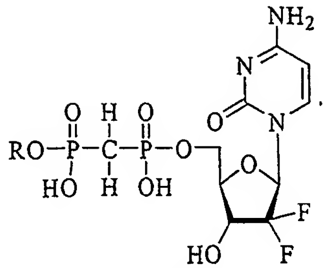


(72)

[0087] Alternatively, the prodrugs of certain nucleotide mimics can be prepared from nucleosides. For example, 2'-deoxy-2',2'-difluorocytidine **73** was converted to the β-*O*-(*S*-pivaloyl-2-thioethyl)-, di-*O*-(*S*-pivaloyl-2-thioethyl)- and tri-*O*-(*S*-pivaloyl-2-thioethoxy)methylenediphosphonate **75-77** in a one-pot reaction. The nucleoside **73** was first reacted with methylenediphosphonotetrachloridate to yield the trichloro intermediate **74**, which was then treated with excess *S*-pivaloyl-2-thioethanol. The resulting three *S*-pivaloyl-2-thioethyl derivatives **75-77** were separated on a reverse-phase HPLC.



(73) **(74)**



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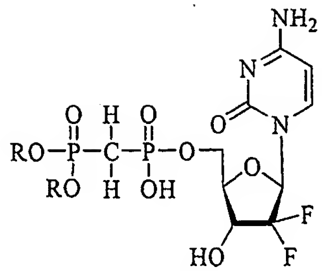

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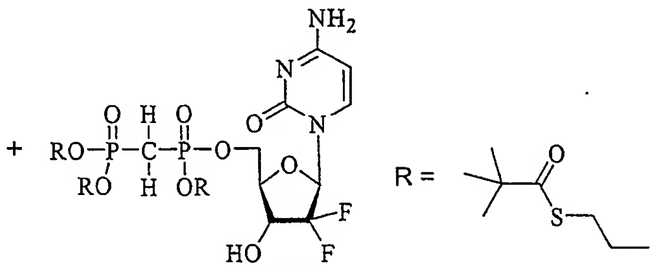

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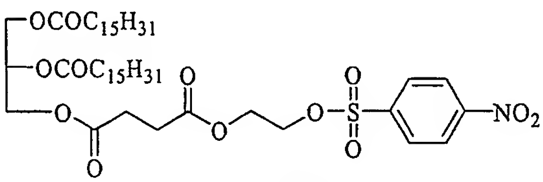
[0088] Another type of prodrugs of the nucleoside mimics is lipid-masked nucleotide mimics, in which a lipid is attached to the terminal phosphorus of a nucleotide mimic directly or through a biologically-cleavable linker. For example, compound **79** was prepared through the condensation of the nucleoside triphosphate mimic **27** with the lipid reagent **78**, which was synthesized from the 1,2-*O*-dipalmitoylglycerol.



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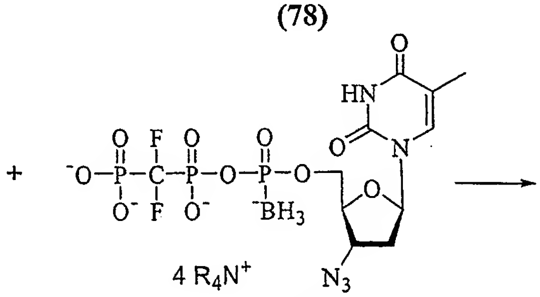

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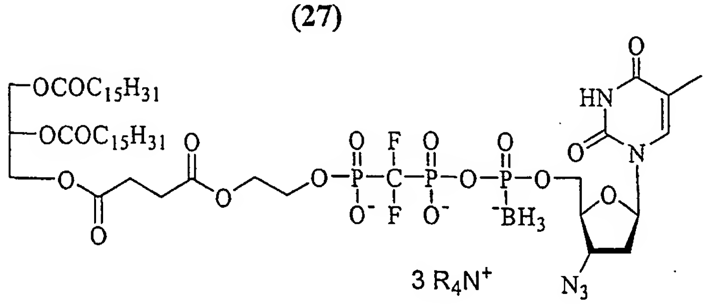

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C. Biological applications and administration

[0089] The nucleoside diphosphate mimics and nucleoside triphosphate mimics of the present invention may be useful for the inhibition of a variety of enzymes including, but not limited to, DNA or RNA polymerases, helicases, ribonucleotide reductases, protein kinases, and telomerases and for the modulation of G-proteins, P2 purinergic receptors and the allosteric sites of a variety of enzymes.

[0090] The nucleotide mimics of the present invention are useful as human therapeutics for the treatment of infectious diseases caused by viruses including, but not limited to, HIV, HBV, HCV, HDV, HSV, HCMV, small pox, West Nile virus, influenza viruses, measles, rhinovirus, RSV. VZV, EBV, vaccinia virus, and papilloma virus.

[0091] The nucleoside diphosphate mimics and nucleoside triphosphate mimics of the present invention are useful for the treatment of infectious diseases caused by bacteria and fungi.

[0092] Those nucleotide mimics that have potent cytotoxicities to fast-dividing cancerous cells are useful for the treatment of proliferative disorders, including, but not limited to, lung cancer, liver cancer, prostate cancer, colon cancer, breast cancer, ovarian cancer, melanoma, and leukemia.

[0093] As the ligands of P2 receptors and G-proteins as well as the inhibitors of protein kinases, the nucleostide mimics of the present invention are useful for the treatment of a wide range of other diseases and disorders such as inflammatory diseases, autoimmune diseases, Type 2 diabetes, and cardiovascular diseases.

[0094] In order to overcome drug resistance, combination therapies are widely used in the treatment of infectious diseases and proliferative disorders. The nucleotide mimics or their prodrugs of the present invention may be therapeutically administered as a single drug, or alternatively may be administered in combination with one or more other active chemical entities to form a combination therapy. The other active chemical entities may be a small molecule, a polypeptide, or a polynucleotide.

[0095] The pharmaceutical composition of the present invention comprises at least one of the compounds represented by Formula **(I)** and **(XVI)** or pharmaceutically acceptable salts, esters or prodrugs thereof as active ingredients. The compositions include those suitable for oral, topical, intravenous, subcutaneous, nasal, ocular, pulmonary, and rectal administration. The

compounds of the invention can be administered to mammalian individuals, including humans, as therapeutic agents.

[0096] For example, the compounds of the invention are useful as antiviral agents. The present invention provides a method for the treatment of a patient afflicted with a viral infection comprising administering to the patient a therapeutically effective antiviral amount of a compound of the invention. The term "viral infection" as used herein refers to an abnormal state or condition characterized by viral transformation of cells, viral replication and proliferation. Viral infections for which treatment with a compound of the invention will be particularly useful include the virues mentioned above.

[0097] A "therapeutically effective amount" of a compound of the invention refers to an amount which is effective, upon single or multiple dose administration to the patient, in controlling the growth of *e.g.*, the microbe or tumor or in prolonging the survivability of the patient beyond that expected in the absence of such treatment. As used herein, "controlling the growth" refers to slowing, interrupting, arresting or stopping the microbial or proliferative transformation of cells or the replication and proliferation of the microbe and does not necessarily indicate a total elimination of *e.g.*, the microbe or tumor.

[0098] Accordingly, the present invention includes pharmaceutical compositions comprising, as an active ingredient, at least one of the compounds of the invention in association with a pharmaceutical carrier. The compounds of this invention can be administered by oral, parenteral (intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection), topical, transdermal (either passively or using iontophoresis or electroporation), transmucosal (*e.g.*, nasal, vaginal, rectal, or sublingual) or pulmonary (e.g., via dry powder inhalation) routes of administration or using bioerodible inserts and can be formulated in dosage forms appropriate for each route of administration.

[0099] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is admixed with at least one inert pharmaceutically acceptable carrier such as sucrose, lactose, or starch. Such dosage forms can also comprise, as is normal practice, additional substances other than inert diluents, *e.g.*, lubricating, agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

[0100] Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, with the elixirs containing inert diluents commonly used in the art, such as water. Besides such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

[0101] Preparations according to this invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilized by, for example, filtration through a bacteria retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions. They can also be manufactured using sterile water, or some other sterile injectable medium, immediately before use.

[0102] Compositions for rectal or vaginal administration are preferably suppositories which may contain, in addition to the active substance, excipients such as cocoa butter or a suppository wax. Compositions for nasal or sublingual administration are also prepared with standard excipients well known in the art.

[0103] Topical formulations will generally comprise ointments, creams, lotions, gels or solutions. Ointments will contain a conventional ointment base selected from the four recognized classes: oleaginous bases; emulsifiable bases; emulsion bases; and water-soluble bases. Lotions are preparations to be applied to the skin or mucosal surface without friction, and are typically liquid or semiliquid preparations in which solid particles, including the active agent, are present in a water or alcohol base. Lotions are usually suspensions of solids, and preferably, for the present purpose, comprise a liquid oily emulsion of the oil-in-water type. Creams, as known in the art, are viscous liquid or semisolid emulsions, either oil-in-water or water-in-oil. Topical formulations may also be in the form of a gel, *i.e.*, a semisolid, suspension-type system, or in the form of a solution.

[0104] Finally, formulations of these drugs in dry powder form for delivery by a dry powder inhaler offer yet another means of administration. This overcomes many of the disadvantages of the oral and intravenous routes.

[0105] The dosage of active ingredient in the compositions of this invention may be varied; however, it is necessary that the amount of the active ingredient shall be such that a suitable dosage form is obtained. The selected dosage depends upon the desired therapeutic effect, on the route of administration, and on the duration of the treatment desired. Generally, dosage levels of between 0.001 to 10 mg/kg of body weight daily are administered to mammals.

[0106] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to prepare and use the compounds disclosed and claimed herein.

Examples

A. Chemical synthesis

[0107] The following examples for the preparation of the nucleotide mimics of the present invention are given in this section. The examples herein are not intended to limit the scope of the present invention in any way. The nucleotide mimics of the present invention can be prepared by those skilled in the art of nucleoside and nucleotide chemistry. The nucleotide mimics of the present invention exist as the salts of nucleotide mimics. However, the amounts of the products given below in the examples are based on UV absorptions and calculated from the molecular weights of H-forms of nucleotide mimics.

Example 1

2'-Deoxy-(*E*)-2'-fluoromethylenecytidine 5'-imidodiphosphate (3)

[0108] Trichloro[(dichlorophosphoryl)imido]phosphorane (83 mg, 0.39 mmol) was added to 2'-deoxy-(*E*)-2'-fluoromethylenecytidine (50 mg, 0.19 mmol) in anhydrous trimethyl phosphate (3 mL) at -15°C under an argon atmosphere. After stirring for 2 h the reaction mixture was poured into ice-cold triethylammonium bicarbonate (TEAB) solution (pH 7.0, 1.0 M, 5 mL) and stirred for 30 minutes. Purification by HPLC gave 9.0 mg of the titled compound **3**.

Example 2

2'-Deoxy-(*E*)-2'-fluoromethylenecytidine 5'-methylenediphosphonate (4)

[0109] Methanephosphonic dichloride (98 mg, 0.39 mmol) was added to 2'-deoxy-(*E*)-2'-fluoromethylenecytidine (50 mg, 0.19 mmol) in anhydrous trimethyl phosphate (3 mL) at -15°C under an argon atmosphere. After stirring for 2 h the reaction mixture was poured into an ice-cold TEAB solution (1.0 M, 5 mL) and stirred for 30 minutes. Purification by HPLC gave 8.6 mg of the titled compound 4.

Example 3

2'-Deoxy-2',2'-difluorocytidine-5'-methylenediphosphonate (5)

[0110] 2'-Deoxy-2',2'-difluorocytidine (0.2 g, 0.76 mmol) was dissolved in anhydrous trimethyl phosphate (2 mL) and stirred with molecular sieves under an argon atmosphere for 16 h. The mixture was cooled to -20°C under an argon atmosphere and methylenebis(phosphonic dichloride) (380 mg, 1.52 mmol) was added. After stirring for 1 h the mixture was quenched using ice-cold TEAB solution (1.0 M, 10 mL) and stirred for 30 minutes. Purification by HPLC gave 28.9 mg of the titled compound.

Example 4

2'-Deoxy-2',2'-difluorocytidine 5'-imidodiphosphate (6)

[0111] 2'-Deoxy-2',2'-difluorocytidine (0.2 g, 0.76 mmol) was dissolved in anhydrous trimethyl phosphate (5 mL) and stirred with molecular sieves under an argon atmosphere for 16 h. The mixture was cooled to -20°C under an argon atmosphere and treated with trichloro-[(dichlorophosphoryl)imido]phosphorane (408 mg, 1.52 mmol). After stirring for 1h the mixture was quenched with ice-cold TEAB solution (1.0 M, 10 mL) and stirred for 30 minutes. Purification by HPLC gave 57.0 mg of the titled compound.

Example 5

2'-Deoxy-2',2'-difluorocytidine 5'-(difluoromethylene)diphosphonate (7)

[0112] Step 1. Tetraisopropyl difluoromethylenediphosphonate (2.1 g, 5.51 mmol) was dissolved in methylene dichloride (20 mL) and treated with trimethylsilyl bromide (3.6 mL, 27.6 mmol). The mixture was heated at reflux for 16 h under an argon atmosphere, cooled and then

evaporated *in vacuo*. The residue was coevaporated with carbon tetrachloride (20 mL), resuspended in carbon tetrachloride (20 mL) and added to phosphorus pentachloride (2.9 g, 13.2 mmol). After stirring for 16 h at room temperature under an argon atmosphere difluoromethylenebis(diphosphonic dichloride) was isolated by vacuum distillation (65°C, 0.5 mmHg) and used directly in the next step.

[0113] Step 2. 2'-Deoxy-2',2'-difluorocytidine (276 mg, 1.05 mmol) was dissolved in anhydrous trimethyl phosphate (5 mL) and stirred with molecular sieves under an argon atmosphere for 16 h. The mixture was added to difluoromethylenebis(phosphonic dichloride) (300 mg, 1 mmol) from Step 1 at -40°C under an argon atmosphere. Stirring continued for 2 h, then the reaction mixture was poured into ice-cold TEAB solution (1.0 M, 5 mL) and stirred for 30 minutes. The aqueous portion was purified by HPLC to give 41.8 mg of the titled compound.

Example 6

3'-Deoxy-3'-azidothymidine 5'-(difluoromethylene)diphosphonate (10)

[0114] To a flask containing difluoromethylenediphosphononic acid tri(tetrabutylamomonium) salt (3.855 g, 4.12 mmol) under argon was added a solution of 3'-azido-3'-deoxy-5'-*O*-tosylthymidine (358 mg, 0.85 mmol) in acetonitrile (2.5 mL). The resulting solution was stirred at room temperature for 27 hours, quenched with water (10 mL), and stirred for 5 min. Purification by HPLC gave 160 mg of the titled compound **10**.

Example 7

3'-Azido-3'-deoxythymidine 5'-α-*P*-borano-β-*P*-methyldiphosphate (13)

[0115] To a stirred solution of 3'-azido-3'-deoxythymidine (67 mg. 0.25 mmol) in anhydrous DMF (1 mL) at 0°C under argon were added tributylamine (77 μL, 0.325 mmol) and then bis(diisopropylamino)chlorophosphine (74 mg, 0.275 mmol) in DMF (06 mL) and THF (0.3 mL). The reaction mixture was stirred at 0°C for 3 h and cooled with ice. A solution of tributylamine (0.24 mL, 1.0 mmol) and methyphosphonic acid (72 mg, 0.75 mmol) in DMF (1 mL) was added, and the resulting solution was stirred at room temperature overnight. Borane-diisopropylethylamine complex (1.0 mL) was added and the resulting mixture was stirred for 7 h. The reaction mixture was cooled with ice and quenched by slow addition of water (2 mL).

The mixture was stirred at room temperature overnight, diluted with water (3 mL). Purification by HPLC gave 7.7 mg of the titled compound **13**.

Example 8

3'-Azido-3'-deoxythymidine 5'-α-*P*-borano-β,γ-(difluoromethylene)triphosphate (**27**)

Step A. The preparation of tetraisopropyl difluoromethylenediphosphonate

[0116] To a stirred solution of tetraisopropyl difluoromethylenediphosphonate (4.5 g, 13.07 mmol) in anhydrous THF (20 mL) at -78°C under argon were added sodium bis(trimethylsilyl)amide (1.0 M in THF, 28.7 mL) and N-fluorobenzenesulfonimide (9.89 g, 31.36 mmol). The reaction mixture was stirred at -78°C for 1 h, quenched with saturated aqueous ammonium chloride (20 mL), warmed to room temperature, diluted with ether, washed with 10% aqueous sodium bicarbonate and then with brine, dried over sodium sulfate, and concentrated. Chromatography on silica gel with 5-30% ethyl acetate in hexanes gave 2.18 g of tetraisopropyl difluoromethylenediphosphonate and 0.32 g of tetraisopropyl monofluoromethylenediphosphonate.

Step B. The preparation of difluoromethylenediphosphonic acid bis(tributylammunium) salts

[0117] To a stirred solution of tetraisopropyl difluoromethylenediphosphonate (2.0 g, 5.26 mmol) in anhydrous acetonitrile (30 mL) was added dropwise trimethylsilyl bromide (4.17 mL, 31.58 mmol). The resulting solution was stirred at 40-42°C for 24 h, concentrated to dryness, and coevaporated with anhydrous acetonitrile once. The residue was re-dissolved in an acetonitrile/water mixture, and then coevaporated with DMF. The residue was dissolved in a DMF solution of tributylamine (1.93 g, 2.48 mL, 10.43 mmol), transfered into multiple small flasks, concentrated to dryness, and coevaporated with anhydrous DMF three times. The residue was dried in a vacuum oven at 40°C for 4 h to give a slightly yellow residue (3.35 g).

Step C. The preparation of 3'-azido-3'-deoxythymidine α-*P*-borano-β,γ-(difluoromethylene)triphosphate (27)

[0118] To a stirred solution of 3'-azido-3'-deoxythymidine (800 mg, 3.0 mmol) in anhydrous DMF (6 mL) and pyridine (1.5 mL) at 0°C under argon was added a solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (728 mg, 3.6 mmol) in DMF (3 mL). The reaction mixture was stirred at room temperature for 1 h and cooled with ice. Tributylamine (1.8 mL, 16.5 mmol) was added, followed by addition of difluoromethylenediphosphonic acid bis(tributylammunium) salt (2.28 g, 3.9 mmol) in DMF (6 mL). The reaction mixture was stirred at room temperature for 1 h and cooled with ice. Borane-diisopropylethylamine complex (12 mL) was added and the resulting mixture was stirred at room temperature overnight. The reaction mixture was cooled with ice and quenched by slow addition of water (30 mL). The mixture was stirred at room temperature for 2 h, diluted with water (30 mL), and extracted with chloroform three times. The aqueous solution was loaded on a reverse-phase (C18) HPLC and eluted with TEAA buffer (pH 7.0), and the purified product was desalted by repeated lyophilization. 3'-azido-3'-deoxythymidine α-*P*-borano-β,γ-(difluoromethylene)triphosphate (501 mg, triethylammonium salts, a mixture of two diastereomers) was obtained as a white foam.

[0119] The two diastereoisomers (α-Rp and α-Sp) were separated under the same HPLC conditions to give pure 3'-azido-3'-deoxythymidine α-(*R*)-*P*-borano-β,γ-(difluoromethylene)triphosphate (the one having shorter retention time is designated as isomer 1, the other as isomer 2) and 3'-azido-3'-deoxythymidine α-(*S*)-*P*-borano-β,γ-(difluoromethylene)triphosphate.

Example 9

7-deaza-2'-*C*-methyladenosine 5'-α-*P*-borano-β,γ-(difluoromethylene)triphosphate (22)

Step A. The preparation of 7-deaza-2'-*C*-methyl-2',3'-*O*-N^6-triacetyladenosine (18)

[0120] A solution of 2'-*C*-methyl-7-deazaadenosine (402 mg, 1.43 mmol) and chlorotrimethylsilane 9237 mg, 1.57 mmol) in anhydrous pyridine (7 mL) was stirred at room temperature overnight. Acetic anhydride (1.08 mL, 11.44 mmol) was added, and the resulting mixture was stirred at room temperature for 3 h. Then DMAP (700 mg, 5.72 mmol) and

triethylamine (0.78 mL, 5.72 mmol) were added, and the resulting mixture was heated at 45-50°C overnight. The mixture was cooled, diluted with ethyl acetate, washed with water (2x), with 2N HCl, then with water (2x), then with 10% NaHCO3, dried over sodium sulfate, and concentrated. Chromatography on silica gel with ethyl aceate yielded 7-deaza-2'-C-methyl-5'-O-trimethylsilyl-2',3'-*O*-N^6-triacetyladenosine as a white solid.

[0121] The white solid was dissolved in THF (5 mL) and TBAF in THF (1.0 M, 1.5 mL) was added. The resulting solution stood at room temperature for 2 h and concentrated. Chromatography on silica gel with 5% methanol in methylene chloride gave 211 mg of 7-deaza-2'-C-methyl-2',3'-*O*-N^6-triacetyladenosine as a white solid.

Step B. The preparation of 7-deaza-2'-C-methyladenosine 5'-α-*P*-borano-β,γ-(difluoromethylene)triphosphate (**22**)

[0122] To a stirred solution of 7-deaza-2'-C-methyl-2',3'-*O*-N^6-triacetyladenosine (62 mg. 0.15 mmol) in anhydrous DMF (0.5 mL) and pyridine (0.1 mL) at 0°C under argon was added a solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (39 mg, 0.195 mmol). The reaction mixture was stirred at room temperature for 1 h and cooled with ice. Tributylamine (0.1 mL) was added, followed by addition of difluoromethylenediphosphonic acid bis(tributylammunium) salt (140 mg, 0.24 mmol) in DMF (0.4 mL). The reaction mixture was stirred at room temperature for 1 h and cooled with ice. Borane diisopropylethylamine complex (0.6 mL) was added and the resulting mixture was stirred at room temperature for 6 h, cooled with ice and quenched by slow addition of water (1.5 mL). The mixture was stirred at room temperature for 1 h, diluted with water (5 mL), extracted with chloroform three times, and concentrated to about 2 mL. Aqueous ammonia (30%, 3 mL) was added and the resulting solution stood at room temperature overnight. Ammonia was evaporated and the remaining aqueous solution was loaded on a reverse-phase (C18) HPLC using TEAA buffer (pH 7.0) to yield the titled compound **22**, the isomer 1: 1.37 mg and the isomer 2: 1.98 mg.

Example 10

3'-Azido-3'-deoxythymidine 5'-α-*P*-borano-β,γ-imidotriphosphate (**23**)

[0123] To a stirred solution of 3'-azido-3'-deoxythymidine (134 mg. 0.50 mmol) in anhydrous DMF (1 mL) and pyridine (0.25 mL) at 0°C under argon was added a solution of 2-

chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (101 mg, 0.50 mmol) in DMF. The reaction mixture was stirred at room temperature for 1 h and cooled with ice. Tributylamine (0.3 mL) was added, followed by addition of a solution of methylenediphosphonic acid (88 mg, 0.50 mmol) and tributylamine (0.3 mL) in DMF (0.5 mL). The reaction mixture was stirred at room temperature for 1 h and cooled with ice. Borane diisopropylethylamine complex (2.0 mL) was added and the resulting mixture was stirred at room temperature for 6 h, cooled with ice and quenched by slow addition of water (5 mL), and stirred at room temperature for 3h. The crude was purified on a reverse-phase (C18) HPLC using TEAA buffer (pH 7.0) gave 60.3 mg of the titled compound **23** as a mixture of two diastereoisomers.

Example 11

3'-Azido-3'-deoxythymidine 5'-α-*P*-borano-β,γ-methylenetriphosphate (24)

[0124] 3'-Azido-3'-deoxythymidine (134 mg, 0.5 mmol) was dissolved in 1 mL of anhydrous DMF in a 10 mL flask kept under argon. To this solution was added 0.25 mL of anhydrous pyridine. A freshly prepared solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (101 mg, 0.495 mmol) in anhydrous DMF (0.5 mL) was added *via* syringe. After 1 hour of stirring at room temperature, tributylamine (0.3 mL) was added followed by a mixture of methylenediphosphonic acid (88 mg, 0.495 mmol) and 3 eq. tributylamine in 0.5 mL of anhydrous DMF. The mixture was stirred for 1 hour and 2 mL of borane-diisopropylethylamine complex was added. After 6 hours stirring at room temperature 5 mL of deionized water was added and stirred at room temperature for 3 hour. Purification by HPLC yielded 46 mg of the titled compound **24**.

Example 12

3'-Azido-3'-deoxythymidine 5'-α-*P*-borano-β,γ-(fluoromethylene)triphosphate (25)

Step A. The preparation of fluoromethylenediphosphonic acid bis(tributylammunium) salts

[0125] To a stirred solution of tetraisopropyl fluoromethylenediphosphonate (320 mg, 0.88 mmol) in 1,2-dichloroethane (5 mL) was added dropwise trimethylsilyl bromide (0.70 mL, 5.28 mmol). The resulting solution was stirred at 40-42°C for 24 h and 3 mL of anhydrous toluene

was added. The mixture was concentrated to dryness, and coevaporated with toluene once. The residue was redissolved in DMF (3 mL)/water (2 mL) mixture and concentrated. The residue was mixed with DMF (2 mL) and tributylamine (0.42 mL, 1.76 mmol) and then concentrated to dryness. The residue was coevaporated with anhydrous DMF two times. The resulting residue was dried in a vacuum oven at 30°C overnight to give a slightly yellow residue (460 mg).

Step B. The preparation of 3'-azido-3'-deoxythymidine α-*P*-borano-β,γ-(fluoromethylene)triphosphate (**25**)

[0126] To a stirred solution of 3'-azido-3'-deoxythymidine (78 mg. 0.29 mmol) in anhydrous DMF (1 mL) and pyridine (0.2 mL) at 0°C under argon was added a solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (77 mg, 0.38 mmol). The reaction mixture was stirred at room temperature for 1 h and cooled with ice. Tributylamine (0.18 mL) was added, followed by addition of fluoromethylenediphosphonic acid bis(tributylammonium) salts (250 mg, 0.43 mmol) in DMF (0.6 mL). The reaction mixture was stirred at room temperature for 1 h and cooled with ice. Borane diisopropylethylamine complex (1.20 mL) was added and the resulting mixture was stirred at room temperature for 6 h, cooled with ice and quenched by slow addition of water (3 mL). The mixture was stirred at room temperature overnight, diluted with water (10 mL), and extracted with chloroform three times.

[0127] The separation of the four α-*P*-borano diastereoisomers of the titled compound **25** on a reverse-phase (C18) HPLC using TEAA buffer (pH 7.0) was conducted to give the isomer 1: 3.75 mg; the isomer 2 (containing other isomers): 8.24 mg; the isomer 3 (containing other isomers): 5.76 mg; the isomer 4: 2.55 mg.

Example 13

3'-Azido-3'-deoxythymidine 5'-α-*P*-borano-β,γ-(dichloromethylene)triphosphate (**26**)

Step A. The preparation of dichloromethylenediphosphonic acid bis(tributylammunium) salt

[0128] An aqueous solution of dichloromethylenediphosphonic acid disodium salt (1.0 g, 3.46 mmol) was loaded on a column of DOWEX 50WX8-100 ion-exchange resin and eluted with water. Tributylamine (1.65 mL, 6.92 mmol) was added and the mixture was shaken

vigorously. The resulting solution was concentrated to dryness and coevaporated with anhydrous DMF three times. The residue was dried under vacuum overnight.

Step B. The preparation of 3'-azido-3'-deoxythymidine α-*P*-borano-β,γ-(dichloromethylene)triphosphate (**26**)

[0129] To a stirred solution of 3'-azido-3'-deoxythymidine (89 mg. 0.33 mmol) in anhydrous DMF (1 mL) and pyridine (0.2 mL) at 0°C under argon was added a solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (83 mg, 0.41 mmol). The reaction mixture was stirred at room temperature for 1 h and cooled with ice. Tributylamine (0.2 mL) was added, followed by addition of dichloromethylenediphosphonic acid bis(tributylammunium) salt (430 mg, 0.43 mmol) in DMF (1 mL). The reaction mixture was stirred at room temperature for 1 h and cooled with ice. Borane diisopropylethylamine complex (1.32 mL) was added and the resulting mixture was stirred at room temperature for 6 h, cooled with ice and quenched by slow addition of water (3 mL). The mixture was stirred at room temperature overnight, diluted with water (10 mL), and extracted with chloroform three times. The two α-*P*-borano diastereoisomers were separated on a reverse-phase (C18) HPLC using TEAA buffer (pH 7.0), and the purified products were desalted by repeated lyophilization to give the titled compound **26**; the isomer 1: 8.3 mg and the isomer 2: 11.3 mg.

Example 14

3'-Deoxythymidine 5'-α-*P*-borano-β,γ-(difluoromethylene)triphosphate (**28**)

[0130] 3'-Deoxythymidine (66.3 mg, 0.150 mmol) was dissolved in 0.5 mL of anhydrous DMF in a 10 mL round-bottom flask kept under argon. To this was added 0.1 mL of anhydrous pyridine. A freshly prepared solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (36.4 mg, 0.180 mmol) in anhydrous DMF (0.5 mL) was added via syringe at 0°C. After stirring at room temperature for 1 hour, tributylamine (0.3 mL) was added followed by a solution of difluoromethylenediphosphonic acid bis(tri-n-butylammonium) salt (113.5 mg, 0.195 mmol) in anhydrous DMF (1 mL). The mixture was stirred at room temperature for 1 hour and borane-diisopropylethylamine complex (0.25 mL) was added at 0°C. After stirring at room temperature for 12 hour water (10 mL) was added and the mixture was stirred at room temperature for 30 minutes. Purification by HPLC yielded 4.8 mg of the titled compound **28**.

Example 15

3'-Azido-3'-deoxythymidine 5'-α-*P*-thio-β,γ-(difluoromethylene)triphosphate (**29**)

[0131] 3'-Azido-3'-deoxythymidine (103.8 mg, 0.388 mmol) was dissolved in 1 mL of anhydrous DMF in a 10 mL round-bottom flask kept under argon. To this was added 0.20 mL of anhydrous pyridine. A freshly prepared solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (101.2 mg, 0.495 mmol) in anhydrous DMF (1 mL) was added via syringe at 0°C. After stirring at room temperature for 1 hour, tributylamine (0.3 mL) was added followed by a solution of difluoromethylenediphosphonic bis(tri-n-butylammonium) salt (294 mg, 0.504 mmol) in anhydrous DMF (1 mL). The mixture was stirred at room temperature for 1 hour and sulfur (24.8 mg, 0.776 mmol) was added at 0°C. After stirring at room temperature for 2 hour distilled deionized water (10 mL) was added and the mixture was stirred at room temperature for 30 minutes. Purification by HPLC yielded 112.8 mg of the titled compound **29**.

Example 16

3'-Deoxythymidine 5'-α-*P*-thio-β,γ-(difluoromethylene)triphosphate (**30**)

[0132] 3'-Deoxythymidine (66.3 mg, 0.150 mmol) was dissolved in 0.5 mL of anhydrous DMF in a 10 mL round-bottom flask kept under argon. To this was added 0.1 mL of anhydrous pyridine. A freshly prepared solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (36.4 mg, 0.180 mmol) in anhydrous DMF (0.5 mL) was added via syringe at 0°C. After stirring at room temperature for 1 hour, tributylamine (0.3 mL) was added followed by a solution of difluoromethylenediphosphonic acid bis(tri-n-butylammonium) salt of (113.5 mg, 0.195 mmol) in anhydrous DMF (1 mL). The mixture was stirred at room temperature for 1 hour and sulfur (9.6 mg, 0.300 mmol) was added at 0°C. After stirring at room temperature for 2 hours, distilled deionized water (10 mL) was added and the mixture was stirred at room temperature for 30 minutes. Purification by HPLC yielded 29 mg of the titled compound **30**.

Example 17

3'-Deoxythymidine 5'-α,α-*P*-dithio-β,γ-(difluoromethylene)triphosphate (**31**)

[0133] 3'-Deoxythymidine (66.3 mg, 0.150 mmol) was dissolved in 0.5 mL of anhydrous DMF in a 10 mL round-bottom flask kept under argon. To this was added 0.1 mL of anhydrous pyridine. A freshly prepared solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (36.4

mg, 0.180 mmol) in anhydrous DMF (0.5 mL) was added via syringe at 0°C. After stirring at room temperature for 1 hour, tributylamine (0.3 mL) was added followed by a solution of difluoromethylenediphosphonic acid bis(tri-n-butylammonium) salt (113.5 mg, 0.195 mmol) in anhydrous DMF (1 mL). The mixture was stirred at room temperature for 1 hour and sulfur (9.6 mg, 0.300 mmol) was added at 0°C. After stirring at room temperature for 2 hours, lithium sulfide (137.8 mg, 3.0 mmol) was added. Stirring was continued for 12 hours. Distilled deionized water (10 mL) was added and the mixture was stirred at room temperature for 30 minutes. Purification by HPLC yielded 2.6 mg of the titled compound **31**.

Example 18

3'-Deoxythymidine 5'-α-*P*-borano-α-*P*-thio-β,γ-(difluoromethylene)triphosphate (32)

[0134] 3'-Deoxythymidine (42.0 mg, 0.186 mmol) was dissolved in 0.5 mL of anhydrous DMF in a 10 mL round-bottom flask kept under argon. To this was added 0.1 mL of anhydrous pyridine. A freshly prepared solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (45.1 mg, 0.223 mmol) in anhydrous DMF (0.5 mL) was added via syringe at 0°C. After stirring at room temperature for 1 hour, tributylamine (0.3 mL) was added followed by a solution of difluoromethylenediphosphonic acid bis(tri-n-butylammonium) salt (140.8 mg, 0.242 mmol) in anhydrous DMF (1 mL). The mixture was stirred at room temperature for 1 hour and borane-diisopropylethylamine complex (0.25 mL) was added at 0°C. After stirring at room temperature for 12 hour, lithium sulfide (17.09 mg, 0.372 mmol) was added. Stirring was continued for 1 hour. Distilled deionized water (10 mL) was added and the mixture was stirred at room temperature for 30 minutes. Purification by HPLC yielded 4.2 mg of the titled compound **32**.

Example 19

3'-Azido-3'-deoxythymidine 5'-α-*P*-ethylamino-β,γ-(difluoromethylene)triphosphate (33)

[0135] 3'-Azido-3'-deoxythymidine (133.6 mg, 0.495 mmol) was dissolved in 1 mL of anhydrous DMF in a 10 mL round-bottom flask kept under argon. To this was added 0.25 mL of anhydrous pyridine. A freshly prepared solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (101.2 mg, 0.495 mmol) in anhydrous DMF (0.5 mL) was added via syringe. After 1 hour of stirring at room temperature, tributylamine (0.3 mL) was added followed by a solution of difluoromethylenediphosphonic acid bis(tri-n-butylammonium) salt

(294 mg, 0.5 mmol) in DMF (0.5 mL). The mixture was stirred for 1 hour and 253 mg (1 mmol) of iodine was added. After 3.5 hours stirring at room temperature 2 mL of a 2 M solution of ethylamine in THF was added and stirred at room temperature for 2 hours. Water (5 mL) was added and the mixture was stirred at room temperature overnight. Purification by HPLC yielded 54.2 mg of the titled compound **33**.

Example 20

3'-Azido-3'-deoxythymidine 5'-α-*P*-phenylamino-β,γ-(difluoromethylene)triphosphate (34)

[0136] 3'-Azido-3'-deoxythymidine (103.8 mg, 0.388 mmol) was dissolved in 1 mL of anhydrous DMF in a 10 mL round-bottom flask kept under argon. To this was added 0.20 mL of anhydrous pyridine. A freshly prepared solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (101.2 mg, 0.495 mmol) in anhydrous DMF (1 mL) was added via syringe at 0°C. After stirring at room temperature for 1 hour, tributylamine (0.3 mL) was added followed by a solution of difluoromethylenediphosphonic acid bis(tri-n-butylammonium) salt (294 mg, 0.504 mmol) in anhydrous DMF (1 mL). The mixture was stirred at room temperature for 1 hour and iodine (197.0 mg, 0.776 mmol) was added at 0°C. After stirring at room temperature for 1 hour, aniline (0.554 mL, 3.88 mmol) was added. Stirring was continued for 4 hours. Distilled deionized water (10 mL) was added and the mixture was stirred at room temperature for 30 minutes. Purification by HPLC yielded 16.2 mg of the titled compound **34**.

Example 21

3'-Deoxythymidine 5'-α-*P*-fluoro-β,γ-(difluoromethylene)triphosphate (35)

[0137] 3'-Deoxythymidine (47.0 mg, 0.208 mmol) was dissolved in 0.5 mL of anhydrous DMF in a 10 mL round-bottom flask kept under argon. To this was added 0.125 mL of anhydrous pyridine. A freshly prepared solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (50.6 mg, 0.250 mmol) in anhydrous DMF (0.5 mL) was added via syringe at 0°C. After stirring at room temperature for 1 hour, tributylamine (0.30 mL) was added followed by a solution of difluoromethylenediphosphonic acid bis(tri-n-butylammonium) salt (157.4 mg, 0.270 mmol) in anhydrous DMF (1 mL). The mixture was stirred at room temperature for 1 hour and iodine (105.6 mg, 0.416 mmol) was added at 0°C. After stirring at room temperature for 1 hour, potassium fluoride (120.8 mg, 2.08 mmol) was added. Stirring was continued for 2 hours.

Water (10 mL) was added and the mixture was stirred at room temperature for 30 minutes. Purification bye HPLC yielded 47.3 mg of the titled compound **35**.

Example 22

3'-Azido-3'-deoxythymidine 5'-α-*P*-azido-β,γ-(difluoromethylene)triphosphate (36)

[0138] 3'-Azido-3'-deoxythymidine (40.09 mg, 0.150 mmol) was dissolved in 0.5 mL of anhydrous DMF in a 10 mL round-bottom flask kept under argon. To this was added 0.10 mL of anhydrous pyridine. A freshly prepared solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (33.4 mg, 0.165 mmol) in anhydrous DMF (0.5 mL) was added via syringe at 0°C. After stirring at room temperature for 1 hour, tributylamine (0.21 mL) was added followed by a solution of difluoromethylenediphosphonic acid bis(tri-n-butylammonium) salt (104.8 mg, 0.180 mmol) in anhydrous DMF (1 mL). The mixture was stirred at room temperature for 1 hour and iodine (76.1 mg, 0.300 mmol) was added at 0°C. After stirring at room temperature for 1 hour, sodium azide (97.5 mg, 1.50 mmol) was added. Stirring was continued for 2 hours. Distilled deionized water (10 mL) was added and the mixture was stirred at room temperature for 30 minutes. Purification by HPLC yielded 16.7 mg of the titled compound **36**.

Example 23

3'-Azido-3'-deoxythymidine 5'-α-*O*-methyl-β,γ-(difluoromethylene)triphosphate (37)

[0139] 3'-Azido-3'-deoxythymidine (40.09 mg, 0.150 mmol) was dissolved in 0.5 mL of anhydrous DMF in a 10 mL round-bottom flask kept under argon. To this was added 0.10 mL of anhydrous pyridine. A freshly prepared solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (33.4 mg, 0.165 mmol) in anhydrous DMF (0.5 mL) was added via syringe at 0°C. After stirring at room temperature for 1 hour, tributylamine (0.21 mL) was added followed by a solution of difluoromethylenediphosphonic acid bis(tri-n-butylammonium) salt (104.8 mg, 0.180 mmol) in anhydrous DMF (1 mL). The mixture was stirred at room temperature for 1 hour and iodine (76.1 mg, 0.300 mmol) was added at 0°C. After stirring at room temperature for 1 hour, sodium methoxide (0.343 mL, 1.50 mmol) was added. Stirring was continued for 30 minutes. Distilled deionized water (10 mL) was added and the mixture was stirred at room temperature for 30 minutes. Purification on by HPLC yielded 10.1 mg of the titled compound **37**.

Example 24

3'-Deoxythymidine 5'-α-*O*-phenyl-β,γ-(difluoromethylene)triphosphate (38)

[0140] 3'-Deoxythymidine (66.3 mg, 0.150 mmol) was dissolved in 0.5 mL of anhydrous DMF in a 10 mL round-bottom flask kept under argon. To this was added 0.1 mL of anhydrous pyridine. A freshly prepared solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (36.4 mg, 0.180 mmol) in anhydrous DMF (0.5 mL) was added via syringe at 0°C. After stirring at room temperature for 1 hour, tributylamine (0.3 mL) was added followed by a solution of difluoromethylenediphosphonic acid bis(tri-n-butylammonium) salt (113.5 mg, 0.195 mmol) in anhydrous DMF (1 mL). The mixture was stirred at room temperature for 1 hour and iodine (76.1 mg, 0.3 mmol) was added at 0°C. After stirring at room temperature for 2 hours, sodium phenoxide (87.1 mg, 0.750 mmol) was added. Stirring was continued for 12 hours. Distilled deionized water (10 mL) was added and the mixture was stirred at room temperature for 30 minutes. Purification by HPLC yielded 2.73 mg of the titled compound **38**.

Example 25

3'-Azido-3'-deoxythymidine 5'-α-*P*-borano-β,γ-difluoromethylene-γ-*O*-methyltriphosphate (40)

[0141] A solution of the bis(tetrabutylammonium) salts of 3'-azido-3'-deoxythymidine 5'-α-*P*-borano-β,γ-(difluoromethylene)triphosphate (0.12 mmol), tributylamine (85 μL, 0.36 mmol) and methyl trifluoromethanesulfonate (54 μL, 0.48 mmol) in anhydrous acetonitrile (2 mL) stood at room temperature overnight. The reaction was quenched with water (2 mL) at 0 °C and then most of the acetonitrile was evaporated. The aqueous solution was subject to HPLC purification to give 1.51 mg of the titled compound **40**.

Example 26

3'-Azido-3'-deoxythymidine 5'-α-*P*-borano-β,γ-difluoromethylene-γ-*O*-palmityltriphosphate (41)

[0142] Step A. Palmitol (1.0 g, 4.12 mmol) and DMAP (0.73 g, 5.9 mmol) in DCM (20 mL) was cooled to -20°C under an argon atmosphere. *p*-Nitrobenzenesulfonyl chloride (1.09 g, 4.9 mmol) was added and the mixture was stirred at 5°C under an argon atmosphere for 18 h. The reaction mixture was poured into cold sodium bicarbonate solution (saturated, 20 mL) and the organic layer was washed with citric acid (10%), brine, and dried over $MgSO_4$. The

suspension was filtered, concentrated *in vacuo* and purified on silica gel flash column chromatography eluting with a gradient of 10-50% ether in hexane to give 1.06 g of palmityl 4-nitrobenzenesulfonate.

[0143] Step B. The bis(tetrabutylammonium) salts of 3'-azido-3'-deoxythymidine 5'-α-*P*-borano-ß,γ-(difluoromethylene)triphosphate (183 mg, 0.146 mmol) was coevaporated with anhydrous DMF (3 X 5 mL). For the third coevaporation, approximately half the volume of DMF was removed and the mixture cooled to 0°C under an argon atmosphere. Palmityl 4-nitrobenzenesulfonate (123 mg, 0.29 mmol) was added. After stirring for 1 h at room temperature the mixture was poured into ice-cold TEAB solution (1.0 M, 10 mL) and stirred for 30 minutes. The aqueous portion was purified on reverse-phase (C18) HPLC to give 6.8 mg of the titled compound **41**.

Example 27

3'-Azido-3'-deoxythymidine 5'-α-*P*-borano-ß,γ-difluoromethylene-γ-*O*-phenyltriphosphate (**42**)

[0144] Step A. A slurry of sodium hydride (320 mg, 8.0 mmol) in anhydrous DMF (10 mL) was treated with phenol (0.9 g, 10.0 mmol) dissolved in anhydrous DMF (2 mL) under an argon atmosphere. After stirring for 1 h at room temperature gas evolution had ceased. 15-Crown-5 (1.59 mL, 8.0 mmol) was added and stirring continued for 1 h. This mixture was used immediately in Step 2.

[0145] Step B. The triethylammonium salts of 3'-azido-3'-deoxythymidine 5'-α-*P*-borano-ß,γ-(difluoromethylene)triphosphate (0.21 mmol) was coevaporated with anhydrous DMF (3 X 5 mL). For the third coevaporation, approximately half the volume of DMF was removed and anhydrous methanol (0.3 mL) was added, followed by N,N'-dicyclohexylcarbodiimide (200 mg). After stirring at room temperature under an argon atmosphere for 3 h, the mixture was concentrated *in vacuo* and the above procedure was repeated once more. After removing solvents *in vacuo*, the residue was resuspended in anhydrous DMF (3 mL) and treated dropwise with the phenoxide solution from Step 1. After stirring at room temperature under an argon atmosphere for 4 h the reaction mixture was treated with water (20 mL), adjusted to pH 6 with HCl solution (1.0 M) and extracted with diethyl ether (3 X 20 mL). The aqueous portion was purified on reverse-phase (C18) HPLC to give 41.3 mg of the titled compound **42**.

Example 28

3'-Azido-3'-deoxythymidine 5'-α-*P*-borano-β,γ-diluoromethylene-γ-*O*-(4-nitrophenyl)triphosphate (43)

[0146] The triethylammonium salts of 3'-deoxy-3'-azidothymidine 5'-α-*P*-borano-β,γ-(difluoromethylene)triphosphate (25 μmol) was coevaporated with anhydrous DMF (3 X 2 mL). For the third coevaporation, approximately half the volume of DMF was removed and methanol (30 μL) was added, followed by N,N'-dicyclohexylcarbodiimide (20 mg). After stirring at room temperature under an argon atmosphere for 3 h, the mixture was evaporated *in vacuo* and the above procedure was repeated once. After removing solvents *in vacuo*, the residue was resuspended in anhydrous DMF (2 mL), treated with anhydrous triethylamine (0.09 mL) followed by addition of *p*-nitrophenol (90 mg). After stirring at room temperature under an argon atmosphere for 4 h reaction mixture was treated with water (10 mL), adjusted to pH 6 with HCl solution (0.1 M) and extracted with diethyl ether (3 X 10 mL). The aqueous portion was purified on reverse-phase (C18) HPLC to give 1.8 mg of the titled compound **43**.

Example 29

3'-Azido-3'-deoxythymidine 5'-γ-*P*-methyl-α,β-(difluoromethylene)triphosphate (44)

[GUANGYI: CHECK (44) HAS CHLORO BUT TITLE COMPOUND DOES NOT]

[0147] To a stirred solution of 1,2,4-1H-triazole (18.3 mg, 0.265 mmol.) in anhydrous acetonitrile (0.5 mL) was added triethylamine (26.8 mg, 37 μL, 0.265 mmol.). The solution was cooled to 0°C, and a solution of methylphosphonic dichloride (17.6 mg, 0.133 mmol) in acetonitrile (0.5 mL) was added dropwise. The reaction mixture was kept with stirring for 40 minutes at room temperature and then centrifuged. The supernatant was added to a solution of the tributylammonium salt of 3'-azido-3'-deoxythymidine 5'-(difluoromethylene)diphosphonate (89.8 mg, 0.0884 mmol) in DMF (1 mL). The reaction mixture was kept with stirring for 1.5 hours and then quenched by adding waster (2 mL). Purification by HPLC gave 13.1 mg of the titled compound **44**.

Example 30

3'-Azido-3'-deoxythymidine 5'-γ-*P*-phenyl-α,β-(difluoromethylene)triphosphate (**45**)

[0148] To a stirred solution of 1,2,4-1H-triazole (18.4 mg, 0.267 mmol.) in anhydrous acetonitrile (0.5 mL) was added triethylamine (27 mg, 37.2 μL, 0.267 mmol.). The solution was cooled to 0°C, and phenylphosphonic dichloride (26 mg, 0.134 mmol.) in acetonitrile (0.5 mL) was added dropwise. The reaction mixture was kept with stirring for 40 minutes at 4°C temperature and then centrifuged. The supernatant was added to a solution of the tributylammonium salt of 3'-azido-3'-deoxythymidine 5'-(difluoromethylene)diphosphonate (90.3 mg, 0.089 mmol) in DMF (1 mL). Similar work-up and purification as described for compound **44** gave 12.8 mg of the titled compound **45**.

Example 31

3'-Azido-3',5'-dideoxythymidine 5'-(β,γ-difluoromethylene)triphosphate (**55**)

Step A. The preparation of 1-(3-*O*-acetyl-2,5-dideoxy-5-iodo-*β*-D-*threo*-pentofuranosyl)thymine (**49**)

[0149] To a stirred solution of 1-(2, 5-dideoxy-5-iodo-*β*-D-*threo*-pentofuranosyl)thymine (for preparation see ref. *J. Org. Chem.* 1964, *29*, 2076) (5.1 g, 14.5 mmol) in anhydrous pyridine (50 mL) at 0°C under argon was added acetic anhydride (7.0 ml, 75.4 mmol). The reaction mixture was stirred at room temperature for 17 h. The reaction mixture was cooled with ice bath and quenched by slow addition of water (15 mL). The reaction mixture was stirred for 30 min and concentrated to dryness, and co-evaporated with anhydrous toluene three times. The residue was re-dissolved in dichloromethane and chromatographed on silica gel with 1-3% methanol in ethyl acetate gave 4.4 g of 1-(3-*O*-acetyl-2,5-dideoxy-5-iodo-*β*-D-*threo*-pentofuranosyl)thymine (**49**).

Step B. The preparation of 1-[3-*O*-acetyl-2,5-dideoxy-5-(di-*O*-ethylphosphono)-*β*-D-*threo*-pentofuranosyl]thymine (**50**)

[0150] To a stirred solution of 1-(3-*O*-acetyl-2,5-dideoxy-5-iodo-*β*-D-*threo*-pentofuranosyl) thymine (**49**) (4.4 g, 12.5 mmol) in freshly distilled triethyl phosphite (50 mL) under argon was heated at 180°C for 30 h. The reaction mixture was concentrated to dryness and the residue left

was re-dissolved in dichloromethane and chromatographed on silica gel with 2-4% ethanol in dichloromethane gave 2.4 g of 1-[3-*O*-acetyl-2,5-dideoxy-5-(di-*O*-ethylphosphono)-*β*-D-*threo*-pentofuranosyl]thymine (**50**) as light yellow foam.

Step C. The preparation of 1-[2,5-dideoxy-5-(di-*O*-ethylphosphono)-3-*O*-mesyl-*β*-D-*threo*-pentofuranosyl]thymine (**51**)

[0151] To a stirred solution of 1-[3-*O*-acetyl-2,5-dideoxy-5-(di-*O*-ethylphosphono)-*β*-D-*threo*-pentofuranosyl]thymine (**50**) (1.08 g, 2.68 mmol) in anhydrous ethanol (75 mL) under argon at 0°C was added sodium ethoxide, (0.28 g, 4.03 mmol) in one portion. Reaction mixture was stirred for 2 h at 0°C. Added more sodium ethoxide, (47.5 mg, 0.67 mmol) after it was stirred for 1 h at 0°C. The resulted yellow color solution was neutralized with DOWEX 50WX8-100 ion exchange resin. The resin was removed by filtration and washed with anhydrous ethanol (60 mL). The filtrate was evaporated to dryness and the residue left was re-dissolved in dichloromethane and chromatographed on silica gel with 3-3.5% ethanol in dichloromethane gave 0.62 g of as 1-[2,5-dideoxy-5-(di-*O*-ethylphosphono)-*β*-D-*threo*-pentofuranosyl]thymine.

[0152] To a stirred solution of 1-[2,5-dideoxy-5-(di-*O*-ethylphosphono)-*β*-D-*threo*-pentofuranosyl]thymine (1.3 g, 3.69 mmol) in anhydrous pyridine (30 mL) under argon at 0°C was added 4-(dimethylamino)pyridine (0.225 g, 1.85 mmol) followed by methanesulfonyl chloride (0.42 mL, 5.54 mmol). The reaction mixture was stirred for 13 h at room temperature. The reaction mixture was cooled with ice bath and quenched by slow addition of water (15 mL). The reaction mixture was stirred for 20 min and concentrated to dryness, and co-evaporated with anhydrous toluene three times. The residue was re-dissolved in dichloromethane and chromatographed on silica gel with 2-3% ethanol in dichloromethane gave 1.5 g of 1-[2,5-dideoxy-5-(di-*O*-ethylphosphono)-3-O-mesyl-*β*-D-*threo*-pentofuranosyl]thymine (**51**)

Step D. The preparation of 1-[3-azido-3, 5-dideoxy-5-(di-*O*-ethylphosphono)- *β*-D-*erythro*-pentofuranosyl]thymine (**52**)

[0153] To a stirred solution of 1-[2,5-dideoxy-5-(di-*O*-ethylphosphono)-3-O-mesyl-*β*-D-*threo*-pentofuranosyl]thymine (**51**) (1.5 g, 3.41 mmol) in anhydrous DMF (25 mL) under argon at room temperature was added NaN_3 (0.44 g, 6.8 mmol). Reaction mixture was stirred for 14 h at 80°C. The reaction mixture was filtered and solvents were evaporated. The residue was re-

dissolved in dichloromethane and chromatographed on silica gel with 3-3.5% ethanol in dichloromethane gave 0.73 g of 1-[3,5-dideoxy-3-azido-5-(di-O-ethylphosphono)- β-D-*erythro*-pentofuranosyl]thymine (**52**).

Step E. The preparation of 3'-azido-3',5'-dideoxythymidine-5'-phosphonic acid (53)

[0154] To a stirred solution of 1-[3,5-dideoxy-3-azido-5-(di-*O*-ethylphosphono)- β-D-*erythro*-pentofuranosyl]thymine (**52**) (0.2g, 0.52 mmol) in anhydrous CH_3CN (5.5 mL) under argon was added Me_3SiBr (0.5 mL, 3.65 mmol). The reaction mixture was stirred for 4 h at 40°C. The reaction mixture was concentrated to dryness, and co-evaporated with anhydrous acetonitrile twice. The residue was and then coevapoarted with MeOH three times. The residue left was re-dissolved in water (3.0 mL) and washed with ether two times and combined aqueous solution was freeze-dried to afford 0.181 g of 3'-azido-3',5'-dideoxythymidine-5'-phosphonic acid (**53**) which was used without further purification.

Step F. The preparation of 3'-azido-3',5'-dideoxythymidine 5'-(β,γ-difluoromethylene)-triphosphate (55)

[0155] To a solution of 3'-azido-3',5'-dideoxythymidine-5'-phosphonic acid (**53**) (0.181 g, 0.55 mmol) in water (2.5 mL) was added 1.34 ml of 1M triethylammoniumbicarbonate buffer and resulted solution was lyophilized to afford 263 mg of triethylammonium salt of compound **53**. The triethylammonium salt of compound **53** (69.3 mg, 0.13 mmol) was dissolved in HMPA (2.5 mL) and 1,1'-carbonyldiimidazole (107 mg, 0.65 mmol) was added at room temperature and stirred for 2 h. Methanol (35 μl) was added and stirring was continued for 45 min. difluoromethylenediphosphonic acid bis(tri-n-butylammonium) salt (397 mg, 0.68 mmol) dissolved in HMPA (2.5 mL) was added and the resulting solution was stirred at room temperature for 4 h. The reaction mixture was cooled in ice-bath and ice/water (4 mL) was added. Purification by HPLC gave 16.6 mg of the titled compound **55**.

Example 32

3'-Azido-3',5'-dideoxythymidine 5'-(β,γ-dichloromethylene)triphosphate (56)

[0156] The triethylammonium salt of compound **53** (59.1 mg, 0.11 mmol) was dissolved in HMPA (2 mL) and 1,1'-carbonyldiimidazole (93 mg, 0.57 mmol) was added at room

temperature and stirred for 2 h. Methanol (30 μl) was added and stirring was continued for 55 min. Dichloromethylenediphosphonic acid tri-n-butylammonium salt (288 mg, 0.51 mmol) dissolved in HMPA (2.5 mL) was added with syringe to the solution of activated AZT monophosphate, and the combined solution was stirred at room temperature for 6 h. The reaction mixture was cooled in ice-bath and ice/water (5 mL) was added. Purification by HPLC gave 8.3 mg of the titled compound **56**.

Example 33

3'-Azido-3',5'-dideoxythymidine 5'-β,γ-imidotriphosphate (57)

[0157] The triethylammonium salt of compound **53** (50.0 mg, 0.09 mmol) was dissolved in HMPA (2 mL) and 1,1'-carbonyldiimidazole (106 mg, 0.66 mmol) was added at room temperature and stirred for 2 h. Methanol (35 μL) was added and stirring was continued for 45 min. The tri-n-butylammonium salt of imidodiphosphate (427 mg, 0.46 mmol) dissolved in HMPA (2 mL) was added with syringe to the solution of activated AZT monophosphate, and the combined solution was stirred at room temperature for 4h. The reaction mixture was cooled in ice-bath and water (5 mL) was added. Purification by HPLC gave 30.6 mg of the titled compound **57**.

Example 34

1-[6,6-Difluoro-6-(β,γ-difloromethylene)triphosphono-2,3,5,6-tetradeoxy-α/β-D-allofuranosyl]thymine (62)

Step A. 6-Diethylphosphono-6,6-difluoro-1-*O*-methyl-2,3,5,6-tetradeoxy-D-allofuranose (59)

[0158] To an ice-cold mixture of trifluoromethanesulfonic anhydride (2.43 mL, 14.4 mmol) in anhydrous CH_2Cl_2 (100 mL) under argon was added 2,6-di-*tert*-butyl-4-methylpyridine (2.96 g, 14.4 mmol). The solution was cooled to -20°C and 2,3-dideoxy-1-*O*-methyl-D-ribofuranose (for preparation, see for example: *Can. J. Chem.* 1969, 47: 4413) (1.9 g, 14.4 mmol) in anhydrous CH_2Cl_2 (75 mL) was added dropwise. The reaction mixture was stirred at -15 to -5°C for 45 min, then poured into ice-cold aq. $NaHCO_3$ (1%, 1 L) and vigorously shaken. The layers were separated and aqueous layer extracted with CH_2Cl_2 (2 × 75 mL). Combined extract was

dried (Na_2SO_4), concentrated and rapidly purified on silica gel column using hexanes and hexanes/Et_2O as eluents. Product-containing fractions were concentrated and used immediately for the next step.

[0159] To a solution of diisopropylamine (6.05 mL, 43.2 mmol) and HMPA (7.52 mL, 43.2 mmol) in anhydrous THF (40 mL) at -78°C under argon was added n-butyllithium (27 mL of 1.6 M in hexanes, 43.2 mmol). The mixture was stirred at 0°C for 1 h and then cooled to -78°C. To this solution were added dropwise *via* transfer needles a cold (-78°C) solution of diethyl (difluoromethyl)phosphonate (6.8 mL, 43.2 mmol) in THF (40 mL) and after 30 min a cold (-78°C) solution of the triflate prepared above in THF (90 mL). The reaction mixture was stirred at -78°C for 2 h and then poured into cold (-10°C) sat. aq. NH_4Cl (300 mL), diluted with Et_2O (300 mL) and layers separated. Aqueous layer was further extracted with EtOAc (2 × 150 mL), combined extract dried (Na_2SO_4) and concentrated. Silica gel chromatography with hexanes and hexanes/EtOAc (10:1) as eluents yielded the titled compound **59** as colorless oil (1.33 g; 31% for 2 steps).

Step B. 1-[6-Diethylphosphono-6,6-difluoro-2,3,5,6-tetradeoxy-α/β-D-allofuranosyl]thymine (60)

[0160] The product **59** from Step A (1.33 g, 4.4 mmol) in acetonitrile (30 mL) was added to the solution of bis(trimethylsilyl)thymine in acetonitrile. The latter was prepared by refluxing thymine (1.11 g, 8.8 mmol) with bis(trimethyl)silyl acetamide (2.3 mL; 8.8 mmol) in acetonitrile (30 mL) for 15 min. The combined mixture was cooled to 0°C when $SnCl_4$ (4.4 mL of 1M in CH_2Cl_2, 4.4 mmol) was added dropwise, and then heated at 75°C for 45 min. After cooling in an ice-bath it was poured into ice-cold aq. $NaHCO_3$ (5%, 200 mL) and extracted with CH_2Cl_2 (200 + 2 × 100 mL). The combined extract was dried (Na_2SO_4), concentrated and purified on silica gel with CH_2Cl_2/MeOH (50:1) as eluent to yield the titled compound **60** as mixture of diastereomers (0.8 g, 46%; α/β 3/2).

Step C. 1-[6,6-Difluoro-6-phosphono-2,3,5,6-tetradeoxy-α/β-D-allofuranosyl]thymine (61)

[0161] To an ice-cold mixture of the product **60** from Step B (550 mg, 1.6 mmol) in anhydrous acetonitrile (15 mL) under argon was added dropwise TMSBr (4.2 mL, 20 equiv).

The resulting mixture was stirred at room temperature for 1 day. The volatiles were removed *in vacuo* under anhydrous conditions and the residue coevaporated several times with toluene and finally partitioned between Et_2O (20 mL) and water (100 mL). Aqueous layer was neutralized with TEAB buffer (1M, pH 8.5) washed with Et_2O (2 × 20 mL) and evaporated to give the triethylammonium salt of **61** as a white solid (630 mg; 76%).

Step D. 1-[6,6-Difluoro-6-(β,γ-difluoromethylenetriphosphono)-2,3,5,6-tetradeoxy-α/β-D-allofuranosyl]thymine (62)

[0162] To a solution of product **61** from Step C (94 mg, 0.17 mmol) in HMPA (2.5 mL) under argon was added 1,1'-carbonyldiimidazole (110 mg, 4 equiv). The reaction mixture was stirred at room temperature for 4 h, when bis[tri(n-butyl)ammonium] (difluoromethylene)bisphosphonate (400 mg, 4 equiv) in HMPA (2 mL) was added. After 8 h (24% of the target triphosphate in mixture with 56% of the imidazolyl phosphonate intermediate as judged by LCMS) the reaction was quenched by pouring into 1M TEAB buffer (10 mL, pH 8.5) and the mixture purified by HPLC to yield 9.2 mg of the titled compound **62**.

Example 35

1-[6,6-Difluoro-6-(β,γ-imidotriphosphono)-2,3,5,6-tetradeoxy-α/β-D-allofuranosyl]-thymine (63)

[0163] To a solution of product **61** from Step C (94 mg, 0.17 mmol) in HMPA (2.5 mL) under argon was added 1,1'-carbonyldiimidazole (CDI) (138 mg, 5 equiv). After 4 h stirring at room temperature the excess of CDI was quenched with MeOH (27.5 μL, 4 equiv). Tetrakis[tri(n-butyl)ammonium)] imidodiphosphate (270 mg, 1.2 equiv) in HMPA (2 mL) was added and stirring at room temperature continued. After 18 h (25% of the target triphosphate in mixture with 41% of the imidazolyl phosphonate intermediate as judged by LCMS) the reaction mixture was quenched by pouring into 1M TEAB buffer (10 mL, pH 8.5) and the mixture purified by HPLC to yield 7.3 mg of the titled compound **63**.

Example 36

1-[6,6-Difluoro-2,3,5,6-tetradeoxy-6-triphosphono-α/β-D-allofuranosyl]thymine (64)

[0164] Following the procedure for the compound **63**, starting from 102 mg (0.19 mmol) of the product **61** from Step C, using tri(n-butyl)ammonium pyrophosphate (246 mg), after 18 h (25% of the target triphosphate in mixture with 41% of the imidazolyl phosphonate intermediate as judged by LCMS) 12 mg of the titled compound **64** was isolated.

Example 37

3'-Azido-3'-deoxythymidine 5'-α, β, β-*O*-(tripivaloyloxymethyl-β,γ-imidodiphosphate (68)

Step A. The preparation of iodomethyl pivalate

[0165] Chloromethyl pivalate (26 g, 25 mL, 0.17 mol) was added dropwise to a stirred solution of sodium iodide (52 g, 0.34 mol) in anhydrous acetone (170 mL) under argon. After stirring at room temperature for 24 h, 170 mL hexanes was added. The precipitated salt was filtered and washed with hexanes. The filtrate was concentrated and dissolved in 170 mL of hexanes, washed with 5% $NaHSO_3$, then water 2 times, and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to yield a yellow liquid. After a flash chromatography on silica with hexanes fractions containing iodomethyl pivalate was combined and washed with $NaHSO_3$ to eliminate I_2. After drying and evaporation 34 g of iodomethyl pivalate were obtained as a slightly-yellow syrup.

Step B. 3'-Azido-3'-deoxythymidine 5'-α, β, β-*O*-(tripivaloyloxymethyl-β,γ-imidodiphosphate (68)

[0166] A mixture of 3'-azido-3'-deoxythymidine 5'-imidodiphosphate (5'-*O*-imidodiphosphate of AZT was prepared according to the literature. (Ma *et al., J. Med. Chem.* **1992**, *35*, 1938-1941) (H+ form, 57 mg, 0.13 mmol) and tributylstannyl methoxide (125 mg, 0.39 mmol) in methanol (3 mL) was stirred at 25°C for 30 min. Methanol was removed by evaporation. To thoroughly remove methanol, the residue was coevaporated with acetonitrile three times. To the residue in acetonitrile (3 mL) were added tetrabutylammonium bromide (126 mg, 0.39 mmol) and iodomethyl pivalate (472 mg, 1.95 mmol). The mixture was refluxed for 1 h and then cooled to 25°C. The mixture was concentrated to a small volume (0.3 mL) under

reduced pressure and then applied on a silica gel column. The column was eluted with chloroform and methanol to give 57 mg of the titled compound **68**.

Example 38

3'-Azido-3'-deoxythymidine 5'-α-*P*-borano-γ-*O*-(pivaloyloxymethyl)-β,γ-(difluoromethylene)triphosphate (69)

[0167] To a stirred solution of the triethylammonium salt of 3'-azido-3'-deoxythymidine 5'-α-*P*-borano-β,γ-(difluoromethylene)triphosphate (**27**) (186 mg, 0.196 mmol) in methanol (5 mL) under argon was added tributylstannyl methoxide (63 mg, 57 μL, 0.196 mmol). The reaction mixture was stirred for 45 minutes, concentrated, coevaporated with acetonitrile (3X3 mL), and dried under high vacuum. The residue was dissolved in acetonitrile (5 mL) under argon, to this solution were added tetrabutylammonium bromide (63.3 mg, 0.196 mmol) and POM (30.7 μL, 0.196 mmol). The reaction mixture was kept with stirring at room temperature for 5.5 hours, quenched by adding water (1 mL), and purified on reverse-phase HPLC to give 4.4 mg of the titled compound **69**.

Example 39

3'-Azido-3'-deoxythymidine 5'-α-*P*-borano-γ-*O*-(*S*-pivaloyl-2-thioethyl)-β,γ-(difluoromethylene)triphosphate (70)

Step A. The preparation of *S*-pivaloyl-2-thioethanol

[0168] To a solution of 2-mercaptoethanol (15.6 g, 14 mL, 0.2 mol) in anhydrous THF (200 mL), NaH (8 g, 0.2 mol, 60% dispersion in mineral oil) was added slowly with magnetic stirring at room temperature. After cooling to 0°C, pivaloyl chloride (28.9 g, 14.8 mL, 0.24 mol) was added dropwise. After 5 hours, it was quenched by adding a mixture of CH_2Cl_2 and water (500 mL, V/V 8:1). The organic layer was separated and washed by water (100 mL) 2 times, and dried by $MgSO_4$. It was filtrated and the solvent was evaporated under reduced pressure. Purification by a flash chromatography on silica with hexanes/EtOAc (10:1) gave 13.7 g of *S*-pivaloyl-2-thioethanol as a light-yellow liquid.

Step B. The preparation of *S*-pivaloyl-2-thioethyl 4-nitrobenzenesulfonate

[0169] 4-Dimethylaminopyridine (937 mg, 7.67 mmol) was added to a stirred solution of *S*-pivaloyl-2-thioethanol (1.038 g, 6.40 mmol) in CH_2Cl_2 (20 mL). Then a solution of 4-nitrobenzenesolfonyl chloride (1.70 g, 7.67 mmol) in CH_2Cl_2 (20 mL) was added dropwise at 0°C under argon. After stirring for 2 hours, the reaction was quenched by adding ice-cooled mixture of water (50 mL) and CH_2Cl_2 (100 mL), and stirred for 5 minutes. It was diluted with CH_2Cl_2 (200 mL), and separated. The organic phase was washed by ice-cooled saturated $NaHSO_4$, brine and dried by $MgSO_4$. Chromatography on silica with 5% EtOAc in hexanes gave 1.09 g of *S*-pivaloyl-2-thioethyl 4-nitrobenzenesulfonate.

Step C. 3'-Azido-3'-deoxythymidine 5'-α-*P*-borano-γ-*O*-(*S*-pivaloyl-2-thioethyl)-β,γ-(difluoromethylene)triphosphate (70)

[0170] To a solution of the bis(tetrabutylammonium salt of 3'-azido-3'-deoxythymidine 5'-α-*P*-borano-β,γ-(difluoromethylene)triphosphate (**27**) (0.107 mmol) in anhydrous acetonitrile was added S-pivaloyl-2-thioethyl 4-nitrobenzenesulfonate (74 mg, 0.214 mmol) in acetonitrile (0.5 mL). The resulting solution stood at room temperature overnight, cooled with ice, quenched with water (3 mL). Most of the acetonitrile was evaporated and the remaining aqueous solution was subject to a reverse-phase HPLC purification to give 5.9 mg of the titled compound **70**.

Example 40

3'-Azido-3'-deoxythymidine 5'-β,β-*O*-di(*S*-pivaloyl-2-thioethyl)imidodiphosphate (71) and 3'-azido-3'-deoxythymidine 5'-α, β, β-*O*-tri(*S*-pivaloyl-2-thioethyl)imidodiphosphate (72)

[0171] To a solution of 3'-azido-3'-deoxythymidine 5'-*O*-imidodiphosphate (128 mg, 0.3 mmol) in anhydrous pyridine (8 mL) at room temperature under argon were added S-pivaloyl-2-thioethanol (487 mg, 10 eqs.) and 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (444 mg, 5 eqs.). After stirring at room temperature for 2 days, the reaction mixture was neutralized with 1 M aqueous triethylammonium hydrogencarbonate buffer (pH=7.5) and extracted with chloroform. The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness under reduced pressure. The crude product was purified by reversed-phase HPLC (C18) to give 1.7 mg of **71** and 3.48 mg of **72**.

Example 41

2'-Deoxy-2',2'-difluorocytidine 5'-β-*O*-(*S*-pivaloyl-2-thioethyl) -α,β-methylene-diphosphonate; 2'-deoxy-2',2'-difluorocytidine 5'-di-α, β–*O*-(*S*-pivaloyl-2-thioethyl)- α,β-methylenediphosphonate; and 2'-deoxy-2',2'-difluorocytidine 5'-tri-*O*-(*S*-pivaloyl-2-thioethyl)-α,β-methylenediphosphonate (75, 76 and 77)

[0172] 2'-Deoxy-2',2'-difluorocytidine (0.5 g, 1.9 mmol) was dissolved in anhydrous trimethyl phosphate (10 mL) and stirred with molecular sieves under an argon atmosphere for 16 h. The mixture was cooled to -20°C under an argon atmosphere and treated with methylenebis(phosphonic dichloride) (522 mg, 2.09 mmol). After stirring for 1 h a white precipitate developed. Tributylamine (6.8 mL, 29 mmol) was added followed by *S*-pivalyl-2-thioethanol (3.1 g, 19 mmol) and the mixture was stirred at room temperature for 1 h. The mixture was quenched using ice-cold TEAB solution (1.0 M, 15 mL) and stirred for 30 minutes. Purification by reverse-phase (C18) HPLC gave 36.5 mg of (**75**), 42.6 g of (**76**); and 64.3 g of (**77**).

Example 42

3'-Azido-3'-deoxythymidine 5'-α-*P*-borano-γ-*O*-[(2,3-*O*-dipalmitoylglyceroxy)carbonyl-3-propionoxy-]2-ethyl-β,γ-(difluoromethylene)triphosphate (79)

Step A. [(2,3-*O*-Dipalmitoylglyeroxy)carbonyl-3-propionoxy]-2-ethanol

[0173] A solution of 1,2-dipalmitoyl-rac-glyceral (844 mg, 1.49 mmol) in methylene chloride (15 mL) was added to a solution of succinyl chloride (1.38 g, 982 μL, 8.91 mmol) in methylene chloride (4 mL) at 0°C under argon. Pyridine (940 mg, 961ul, 11.9 mmol) was then added in five-portions with a 10 min interval between two additions. The reaction continued for 1.5 hours, then cooled down to -78°C. Ethylene glycol (1.85 g, 1.66 mL, 29.7 mmol) was added, and the resulting mixture warmed up to room temperature and stirred for 2.5 hours. A mixture of EtOAc (200 mL) and water (200 mL) was added, the aqueous layer was extracted with EtOAc. The combined organic phase was washed by water, saturated $NaHCO_3$ (2 times), dried over $MgSO_4$. Chromatography on Silica gel with 10-20% EtOAc in hexane yielded 0.68 g of [(2,3-*O*-Dipalmitoylglyeroxy)carbonyl-3-propionoxy]-2-ethanol.

Step B. [(2,3-*O*-Dipalmitoylglyeroxy)carbonyl-3-propionoxy]-2-ethyl *p*-nitrobenzenesulfonate (78)

[0174] In a stirred solution of [(2,3-*O*-dipalmitoylglyeroxy)carbonyl-3-propionoxy]-2-ethanol (516 mg, 0.685 mmol) in methylene chloride (5 mL) under argon, a 4-dimethylaminopyridine (210 mg, 1.72 mmol) was added. The solution was cooled to 0°C and a solution of 4-nitrobenzenesulfonyl chloride (310 mg, 1.40 mmol) in methylene chloride (7 mL) was added. The reaction mixture was stirred at 0°C for 0.5 h, warmed to room temperature. After stirring for another hour, the reaction was quenched by adding ice-cooled water (6 mL) and stirred for 5 minutes. The mixture was diluted with methylene chloride (200 mL), and washed with saturated ice-cooled $NaHCO_3$ 2 times (200 mL each), then ice-cooled water 2 times (200 mL each), dried over $MgSO_4$, purified by chromatography on silica with 20% EtOAc in hexanes to give 0.544 g of the titled product (**78**).

Step C. 3'-Azido-3'-deoxythymidine 5'-α-*P*-borano-γ-*O*-[(2,3-*O*-dipalmitoylglyceroxy)carbonyl-3-propionoxy]-2-ethyl)-β,γ-(difluoromethylene)triphosphate (79)

[0175] To a stirred solution of [(2,3-*O*-dipalmitoylglyeroxy)carbonyl-3-propionoxy]-2-ethyl *p*-nitrobenzenesulfonate (**78**) (480 mg, 0.527 mmol) in $CHCl_3$ (2.5 mL) under argon, tris(tetra-*n*-butylammonium) salt of 3'-azido-3'-deoxythymidine 5'-α-*P*-borano-β,γ-(difluoromethylene)triphosphate (**27**) (163 mg, 0,13 mmol) in $CHCl_3$ (1.8 mL) was added, followed by addition of tributylamine (185 mg, 62 μL, 0.259 mmol). The reaction mixture was stirred for 24 hours, extracted with 50% water/acetonitrile (3 x 6 mL), filtered, and concentrated to remove partial acetonitrile. The aqueous mixture was purified by reverse-phase HPLC to give 20 mg of the titled compound **79**.

[GUANGYI: THERE IS NO COMPOUND (80) AND (81) IN SCHEMES, WHICH IS NOT A PROBLEM BUT JUST CHECKING THAT SCHEMES WERE NOT INADVERTENTLY OMITTED.)

Example 43

(*E*)-2'-Deoxy-2'-(fluoromethylene)cytidine 5'-ethylenediphosphonate (80)

[0176] Ethylenediphosphonic acid (100 mg, 0.53 mmol) was treated with oxalyl chloride (2 mL, as solvent) and anhydrous DMF (0.1 mL, cat.). After heating at reflux for 10 minutes under an argon atmosphere a solution was obtained and after a further 3 h the reaction mixture was cooled to room temperature and concentrated *in vacuo*. The residue was coevaporated with anhydrous acetonitrile (3 X 10 mL). The residue was dissolved in anhydrous trimethyl phosphate (2 mL), cooled to -15°C under an argon and (*E*)-2'-deoxy-2'-(fluoromethylene)cytidine (64 mg, 0.25 mmol) was added. After stirring for 2 h the reaction mixture was quenched with ice-cold TEAB solution (1.0 M, 5 mL) and stirred for 30 minutes. Purification by HPLC gave 2.9 mg of the titled compound **80**.

Example 44

2'-Deoxy-2',2'-difluorocytidine 5'-ethylenediphosphonate (81)

[0177] Ethylenediphosphonic acid (173 mg, 0.91 mmol) was treated with oxalyl chloride (2 mL, as solvent) and anhydrous DMF (0.1 ml, cat.). After heating at reflux for 10 minutes under argon, a solution was obtained and after a further 3 h the reaction mixture was cooled to room temperature and evacuated *in vacuo*. The residue was co-evaporated with anhydrous acetonitrile (3X10 ml). The residue was dissolved in anhydrous trimethyl phosphate (2 mL), cooled to -15°C and 2'deoxy-2',2'-difluorocytidine (93 mg, 0.46 mmol) was added under argon. After stirring for 2 h the reaction mixture was quenched with ice-cold TEAB solution (1.0 M, 5 mL) and stirred for 30 minutes. Purification by HPLC gave 7.8 mg of the titled compound **81**.

B. Biological assays

Example 45

HIV Reverse Transcriptase inhibition assays

[0178] The assays employed to measure inhibition of HIV Reverse Transcriptase (HIV RT) catalyzed/RNA-directed DNA polymerization are described below. The effectiveness of the compounds of the present invention as inhibitors of HIV RT-catalyzed DNA polymerization was measured in the following assays.

A. Assay for Inhibition of HIV Reverse Transcriptase-catalyzed DNA Polymerization (Homopolymer RNA Template).

[0179] This assay was used to measure the ability of the nucleotide mimics of the present invention to inhibit the enzymatic synthesis of complementary strand DNA from a DNA-primed template of homopolymeric RNA. This assay is a modification of a published procedure (Seville, *et al.*, "Fluorometric Assay for DNA Polymerases and Reverse Transcriptase", *BioTechniques* 1996, 21, 664-72).

Procedure:

Assay Buffer Conditions: (50 μL-total/reaction)

50 mM Tris-HCl, pH 8.1

6.5 mM MgCl2

100 mM NaCl

10 mM DTT

5 μM dTTP (thymidine triphosphate)

1 ug/mL Primed-poly(A) RNA

2 nM purified HIV Reverse Transcriptase (Type B, 66 kDa subunit)

[0180] The compounds were tested at various concentrations up to 500 μM final concentration. DNA polymerase activity was measured in a reaction buffer containing primed-RNA template and dTTP diluted to appropriate concentrations in assay buffer. Nucleotide mimics of the present invention were diluted in buffer and pipetted into the wells of a 96-well plate. The reaction was initiated by addition of enzyme and allowed to proceed at 37°C for 10 minutes. The reaction was quenched by addition of 5 μL 0.2 M EDTA, pH 8.0.

[0181] Blank reactions were prepared in parallel with the test reactions in which either enzyme or dTTP was omitted from the reactions, substituted by an appropriate volume of enzyme diluent or assay buffer, respectively.

[0182] 200 µL of diluted PicoGreen dsDNA Quantitation Reagent (Molecular Probes, Inc, Eugene, OR) was added to each well of a 96-well plate and incubated at room temperature for 5 minutes. Plate wells were read on a microplate fluorometer (Molecular Devices Corp., Sunnyvale, CA). The wells were excited at 480 nm and the fluorescence emission intensity (RFU) was measured at 520 nm. The percentage of inhibition was calculated according to the following equation:

% Inhibition = [1-(RFU in test reaction – RFU in blank) / (RFU in control reaction – RFU in blank)] x 100.

[0183] Inhibition constants (K_i) were determined for representative compounds that exhibited ≥ 50% inhibition at 10 µM when tested in the HIV RT homopolymer polymerization assay. Each inhibitor was titrated over an appropriate range of concentrations, and inhibition constants were determined using the following equations:

Michaelis-Menten equation:

$$v = V_m [S] / (K_m + [S])$$

Competitive inhibition equation:

$$v = V_m [S] / (K_m (1 + [I] / K_i) + [S])$$

where v = initial velocity, V_m = maximal velocity, S = substrate, I = inhibitor, K_m = Michaelis constant, and K_i = inhibition constant.

[0184] Representative compounds of the present invention tested in the HIV RT-catalyzed DNA polymerization assay exhibited inhibition constants less than 100 µM.

B. Assay for Inhibition of HIV Reverse Transcriptase-catalyzed DNA Polymerization (Heteropolymer RNA Template).

[0185] Certain compounds of the present invention were also tested to determine their ability to inhibit HIV RT-catalyzed DNA polymerization on a heteropolymeric primed-RNA template in the presence of all four natural deoxynucleotide triphosphate (dNTP) substrates. This assay is a modification of a published procedure (Parker *et al.*, "Mechanism of Inhibition of

Human Immunodeficiency Virus Type 1 Reverse Transcriptase and Human DNA Polymerases α, β, and γ by the 5'-Triphosphates of Carbovir,3'-Azido-3'-deoxythymidine,2'-3'-Dideoxyguanosine,and3'-Deoxythymidine", *J. Biol. Chem.* **1991**, 266 (3), 1754-62).

Procedure:

Assay Buffer Conditions: (120 µL-total/reaction)

50 mM Tris-HCl, pH 8.1
6.5 mM $MgCl_2$
100 mM NaCl
10 mM DTT
50 µM dNTPs (dATP, TTP, dGTP, dCTP)
5 ug/mL Primed-Ribosomal RNA (*E. Coli*)
10 Units HIV Reverse Transcriptase (purified, Type B, 66 kDa subunit)

[0186] HIV RT DNA polymerase activity was measured in a reaction buffer containing primed-ribosomal RNA template and dNTPs diluted to appropriate concentrations in assay buffer and pipetted into 1.5 mL microcentrifuge tubes. Nucleotide mimics of the present invention were diluted in buffer and tested at various concentrations up to 5 µM final concentration. The reaction was initiated by addition of enzyme and allowed to proceed at 42°C for 60 to 90 minutes. The reaction was quenched by addition of 12 µL 0.2 M EDTA, pH 8.0.

[0187] Blank reactions were prepared in parallel with the test reactions in which either enzyme or dNTP was omitted from the reactions, substituted by an appropriate volume of enzyme diluent or assay buffer, respectively.

[0188] 50 µL each of reaction was transferred to the well of a 96-well plate (in duplicate). 200 µL of diluted PicoGreen dsDNA Quantitation Reagent was added to each well of a 96-well plate and incubated at room temperature for 5 minutes. Plate wells were read on a microplate fluorometer. The wells were excited at 480 nm and the fluorescence emission intensity (RFU) was measured at 520 nm. The percentage of inhibition and inhibition constants (K_i) were determined for representative compounds as described above.

Table 1.
Inhibition of HIV Reverse Transcriptase by Nucleotide Mimics

Compound	K_i *(µM)*
8	4.4

17-isomer 1	0.009
17-isomer 2	0.048
27-isomer 1	0.008
27-isomer 2	0.061
26-isomer 1	0.093
26-isomer 2	0.074
23	0.105
25	0.027
29	0.090
42	0.113
40	0.069

Example 46

Ribonucleotide reductase inhibition assay

[0189] The assays employed to measure inhibition of Ribonucleotide Reductase (RNR) activity are described below. The effectiveness of the compounds of the present invention as inhibitors of RNR enzyme was determined using the following assays.

A. Assay for Inhibition of RNR Activity

[0190] This assay was used to measure the ability of the nucleotide mimics of the present invention to inhibit the enzymatic reaction catalyzed by RNR enzyme. In the reduction of cytidine 5'-diphosphate (CDP) to 2'-deoxycytidine 5'-diphosphate (dCDP), stoichiometric amounts of oxidized thioredoxin are formed. This protein is reduced by NADPH in a reaction catalyzed by thioredoxin reductase. By coupling the two reactions, RNR activity was measured by spectrophotometric determination of NADPH oxidation at 340 nm using a UV/VIS spectrophotometer. This assay is a modification of a published procedure (van der Donk *et al.*, "Inactivation of Ribonucleotide Reductase by *(E)*-2'-Fluoromethylene-2'-deoxycytidine 5'-Diphosphate: A Paradigm for Nucleotide Mechanism-Based Inhibitors," *Biochemistry* 1996, 35, 8381-91).

Procedure:

Irreversible Inactivaton Conditions: (50 μL-total/reaction)

50 mM HEPES, pH 7.6
15 mM $MgSO_4$
1 mM EDTA
1.6 mM ATP
0.5 mM NADPH

20 μM Thioredoxin
0.5 μM Thioredoxin Reductase
1 μM RNR R1
2 μM RNR R2

Assay Conditions: (200 μL-total/reaction)
50 mM HEPES, pH 7.6
15 mM $MgSO_4$
1 mM EDTA
1.6 mM ATP
0.5 mM NADPH
20 μM Thioredoxin
0.5 μM Thioredoxin Reductase
1 mM CDP

[0191] The compounds were tested at various concentrations up to 500 μM final concentration. The nucleotide mimics of the present invention were added to irreversible inactivation mixtures at the desired concentrations. An identical control was run in which the inhibitor was replaced with H_2O. After a 3-minute incubation, 20 μL was removed from the inactivation mixtures and assayed for activity.

[0192] The standard RNR assay is performed in a 96-well plate (Corning). Aliquots from the inactivation mixtures were added to an appropriate volume of assay buffer pipetted into the plate wells. The reactions were initiated by the addition of the substrate CDP. The reactions were allowed to proceed for 5 minutes at 25°C. The oxidation of NADPH was monitored at 340 nm on a microplate spectrophotometer (Molecular Devices Corp, Sunnyvale, CA). Initial velocity data (mA min^{-1}) was collected and fit to the equations below.

[0193] Blank reactions were prepared in parallel with the test reactions in which substrate was omitted from the reactions, substituted by an appropriate volume of H_2O.

[0194] The <u>percentage of inhibition</u> was calculated according to the following equation:

% Inhibition = [1-(mA min^{-1} in test reaction – mA min^{-1} in blank) / (mA min^{-1} in control reaction – mA min^{-1} in blank)] x 100.

[0195] The inhibitor concentration values giving 50% inhibition (IC_{50}) were determined for representative compounds that exhibited ≥ 50% inhibition at 250 μM when tested in the RNR inhibition assay. Each inhibitor was titrated over an appropriate range of concentrations, and IC_{50} values were determined using the equation:

IC_{50} equation:

$v_i / v_o = 1 / (1 + [I] / IC_{50})$

where v_i = initial velocity in the presence of inhibitor at concentration I, v_o = initial velocity in the absence of inhibitor, and IC_{50} = inhibitor concentration giving 50% inhibition.

Representative compounds of the present invention tested in the RNR inhibition assay exhibited inhibition constants less than 250 μM.

Table 2.
Inhibition of Ribonucleotide Reductase by Nucleotide Mimics

	IC_{50} (μM)
3	8.9
6	12.6

Example 47

Tubulin assays

[0196] The assays employed to measure the polymerization and stability of tubulin microtubule protein are described below. The effectiveness of the compounds of the present invention as modulators of tubulin microtubule protein dynamics was measured in the following assays.

A. Assay for Inhibition of Microtubule Polymerization.

[0197] This assay was used to measure the ability of the nucleotide mimics of the present invention to inhibit the polymerization of tubulin subunits and subsequent assembly of microtubule protein. This assay is a modification of a published procedure (Xu *et al.*, "Interaction of Tubulin with Guanosine 5'-O-(1-Thiotriphosphate) Diastereoisomers: Specificity of the α-Phosphate Binding Region", *Biochemistry* **1994**, 33, 11884-90).

Procedure:

Assay Buffer Conditions: (100 μL-total/reaction)

80 mM PIPES, pH 6.9

2 mM $MgCl_2$

0.5 mM EGTA

10% glycerol

50 μM GTP (guanosine 5'- triphosphate)

300 ug purified bovine brain tubulin (Cytoskeleton, Denver, CO)

[0198] The compounds were tested at various concentrations up to 500 μM final concentration. Polymerization assays were performed in 96-well microplates (Costar), in assay buffer containing GTP and tubulin. The nucleotide mimics of the present invention were diluted in buffer and pipetted into the plate wells. Samples were kept on ice until the reactions were initiated by moving the plates into a 37°C incubator. The polymerization reaction was allowed to proceed at 37°C for 30 minutes, monitored at 340 nm using a microplate spectrophotometer with a temperature-controlled incubator (Molecular Devices, Sunnyvale, CA). Initial velocity data (mA min^{-1}) was collected and fit to the equations below.

[0199] Blank reactions were prepared in parallel with the test reactions in which either tubulin or GTP was omitted from the reactions, substituted by an appropriate volume of assay buffer.

[0200] The <u>percentage of inhibition</u> was calculated according to the following equation:

% Inhibition = [1-(mA min^{-1} in test reaction – mA min^{-1} in blank) / (mA min^{-1} in control reaction – mA min^{-1} in blank)] x 100.

[0201] Inhibition constants (K_i) were determined for representative compounds that exhibited > 50% inhibition at 250 μM when tested in the tubulin polymerization assay. Each inhibitor was titrated over an appropriate range of concentrations, and inhibition constants were determined using the following equations:

<u>Michaelis-Menten equation:</u>

$$v = V_m [S] / (K_m + [S])$$

<u>Competitive inhibition equation:</u>

$$v = V_m [S] / (K_m (1 + [I] / K_i) + [S])$$

where v = initial velocity, V_m = maximal velocity, S = substrate, I = inhibitor, K_m = Michaelis constant, and K_i = inhibition constant.

[0202] Representative compounds of the present invention tested in the tubulin polymerization assay exhibited inhibition constants less than 200 μM.

B. Assay for Disruption of Microtubule Dynamics

[0203] This assay was used to measure the ability of the nucleoside derivatives of the present invention to interfere with normal microtubule protein dynamics, including the rate of polymerization, the rate of depolymerization, and stabilization of microtubule protein. This assay is a modification of the method described in O. Monasterio and S.N. Timasheff, " Inhibition of Tubulin Self-Assembly and Tubulin-Colchicine GTPase Activity by Guanosine 5'-(γ-Fluorotriphosphate)", *Biochemistry* 26: 6091-99 (1987).

Procedure:

Assay Buffer Conditions: (100 μL-total/reaction)

80 mM PIPES, pH 6.9

2 mM $MgCl_2$

0.5 mM EGTA

10% glycerol

300 ug purified bovine brain tubulin (Cytoskeleton, Denver, CO)

[0204] The compounds were tested at various concentrations up to 500 μM final concentration. Polymerization assays were performed in 96-well microplates (Costar), in assay buffer containing tubulin. The nucleotide mimics of the present invention were diluted in buffer and pipetted into the plate wells. Samples were kept on ice until the reactions were initiated by moving the plates into a 37°C incubator. The polymerization reaction was allowed to proceed at 37°C for 30 minutes, monitored at 340 nm using a microplate spectrophotometer with a temperature-controlled incubator (Molecular Devices, Sunnyvale, CA).

[0205] Depolymerization of the microtubule protein was achieved by transferring the plates from 37°C to a 0-4°C incubator for 20 minutes. Plates were then returned to the 37°C incubator, and the second cycle of polymerization was allowed to proceed for 15 minutes. Initial velocity data (mA min^{-1}) for both polymerization cycles was collected and fit to the equations described above.

[0206] Blank reactions were prepared in parallel with the test reactions in which tubulin was omitted from the reactions, substituted by an appropriate volume of assay buffer.

[0207] The degree of disruption of microtubule dynamics was determined relative to control reactions containing 50 μM GTP and tubulin.

C. Phosphate Assay for Tubulin-GTP Hydrolysis

[0208] This assay was used to measure the ability of the nucleotide mimics of the present invention to inhibit hydrolysis of GTP by tubulin. The enzymatic conversion of GTP to GDP + inorganic phosphate (P_i) is monitored in a coupled-spectrophotometric assay using the EnzChek Phosphate Assay Kit (Molecular Probes, Inc, Eugene, OR). This assay is a modification of a published procedure (Vandecandelaere *et al.*, "Phosphate Release during Microtubule Assembly: What Stabilizes Growing Microtubules?", *Biochemistry* **1999**, 38, 8179-88).

Procedure:

Assay Buffer Conditions: (100 µL-total/reaction)

80 mM PIPES, pH 6.9

2 mM $MgCl_2$

0.5 mM EGTA

10% glycerol

50 µM GTP

50 ug purified bovine brain tubulin

[0209] The compounds were tested at various concentrations up to 500 µM final concentration. GTP hydrolysis assays were performed in 96-well microplates (Costar), in assay buffer containing tubulin, GTP, MESG, and PNP. The nucleotide mimics of the present invention were diluted in buffer and pipetted into the plate wells. Samples were kept on ice until the reactions were initiated by moving the plates into a 37°C incubator. The hydrolysis reaction was initiated by addition of GTP, and allowed to proceed at 37°C for 10 minutes. The reaction was monitored at 360 nm using a microplate spectrophotometer with a temperature-controlled incubator (Molecular Devices, Sunnyvale, CA). Initial velocity data (mA min^{-1}) was collected and fit to the equations described above.

[0210] Blank reactions were prepared in parallel with the test reactions in which GTP was omitted from the reactions, substituted by an appropriate volume of assay buffer.

Table 3.
Inhibition of Tubulin Polymerization by Nucleotide Mimics

Compound Name	K_i (μM)
Guanosine 5'-diphosphate	15.3

Guanosine 5'-imidodiphosphate	5.7
2'-Deoxy-2'-fluoromethylenecytidine 5'-diphosphate	19.4
2'-Deoxy-2'-fluoromethylenecytidine 5'-ethylenediphosphonate (**81**)	15.7

Example 48
Mammalian cell growth inhibition assay

[0211] The assays employed for determining the cytotoxicity of the nucleotide mimics of the present invention to mammalian cells are described below.

Mammalian Cells and Growth Conditions

[0212] Human CCRF-CEM cells were obtained from American Tissue Culture Collection (ATCC) and grown according to ATCC specifications. Briefly, CCRF-CEM, a lymphoblastoid cell line, was grown and maintained as a suspension culture in RPMI 1640 medium containing 2 mm L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/L glucose, 1.5 g/L sodium bicarbonate and supplemented with 10% (v/v) dialyzed and heat-inactivated fetal bovine serum. CEM cells were grown at 37°C in a 95% humidified environment and 5% CO_2 atmosphere.

Cytotoxicity Assays: MTT Assay.

[0213] The cytotoxicity of the nucleotide mimics of the present invention to mammalian cells was determined by measuring cell survival using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Slater T.F. *et al., Biochim. Biophys. Acta* **1963**, 77, 383; Mossman T. *J. Immunol. Methods* **1983**, 65, 55; M.E. *et al.,*1999, *J. Biol. Chem.* 28505-13). MTT is a water soluble tetrazolium salt that is converted to an insoluble purple formazan by active mitochondrial dehydrogenases of living cells. Dead cells do not cause this change. Conversion of MTT into the insoluble formazan by non-treated control or treated cells was monitored at 540 nm.

[0214] CCRF-CEM cells (3×10^4) were plated in 96-well plates in RPMI media. The next day, cells were incubated with different concentrations (0-200 μM) of the nucleotide mimics of the present invention for 72 hr. Following treatment, MTT (2mg/ml in PBS) dye was added to each well so that the final concentration was 0.5 mg/ml and then incubated for 4 hr at 37°C.

Media and MTT dye were removed without disturbing the cells and 100% DMSO was added to dissolve the precipitate. After a 10 minute incubation at room temperature, the optical density values were measured at 540 nm,. using the Spectra Max Plus plate reader. Survival was expressed as the percentage of viable cells in treated samples relative to non-treated control cells.

Table 4.
Antiproliferative effect of nucleotide mimics and their prodrugs

Compound	Cytotoxicity IC50 (μM)
Gemcitabine	0.035
5	4.8
75	0.025
76	0.025
77	0.1

Example 49

Antimicrobial assays

[0215] To examine the antimicrobial potential of the nucleotide mimics of the present invention an assay was employed that allowed the screening of a large number of compounds simultaneously. The type of bacteria chosen to screen the compounds are organisms associated with human disease and represent major groups of bacteria based on their structure and metabolism.

Lawn Screening Assay:

[0216] Bacterial cultures of *Escherichia coli* and *Staphylococcus aureus* were incubated overnight at 37°C in a shaker incubator. A lawn of each overnight bacterial culture was made by plating 200μl of bacteria on agar plates containing Nutrient Broth. Immediately after plating, sterile blank paper discs were put on top of the lawn and a compound was applied to each blank paper disc. Plates were then incubated overnight and examined for the inhibition of bacterial growth the following day.

Minimal Inhibitory Concentration Determination

[0217] Bacterial cells (2 x 10^4) growing in exponential phase were plated in 96-well plates and treated with different concentrations (0-200 µg/ml) of the nucleotide mimics of the present invention. The plates were incubated overnight at 37°C and then examined spectrophotometrically at 600 nm to determine the minimum concentration of each compound that inhibited replication of bacteria as determined by no increase in absorbance at 600 nm.

Table 5.
Antibacterial activity of nucleotide mimics and their prodrugs

Compound	E. coli MIC µg/ml (uM)	S. aureus MIC µg/ml (µM)
AZT	2 (7.5 uM)	NA*
27-isomer 1	60 (111)	NA
27-isomer 2	50 (93)	NA
26-isomer 1	12 (21)	NA
26-isomer 2	10 (17.5)	NA
25-isomer 1	25-50 (48-96)	NA
25-isomer 4	25-50 (48-96)	NA
23	50-100 (99-198)	NA
69	0.75 (1)	NA
70	6 (8.8)	NA
71	20 (28)	NA
41	25-50 (33-66)	5-10 (6.5-13)
40	25-50 (40-80)	NA
5	NA	>200 (>475)
75	NA	15 (26)
76	NA	25 (35)

NA*. Not active

Example 50

Serum Stability Assessment

[0218] The stability of nucleotide mimics was assessed in fetal calf serum generally following the procedure outlined by Arzumanov *et al.*, (*J. Biol. Chem.* **1996**, 271(40), 24389-24394). Fetal calf serum purchased from HyClone Corporation was mixed 1:1 with each compound containing Tris-HCl buffer and $MgCl_2$. Typically the total volume used for the experiment was 500 µl. The final concentrations of the reaction components were as follows:

50 mM Tris-HCl, pH 7.4

0.1 mM $MgCl_2$

500 µM nucleotide mimic

50% (v/v) fetal calf serum

[0219] The reaction mixtures were made up and incubated at 37°C. At appropriate times aliquots of 25 μl were removed and added to 65 μl ice-cold methanol. These solutions were incubated for at least one hour at -20°C and typically overnight. After incubation samples were centrifuged for at least 20 minutes at high speed in a microcentrifuge. The supernatant was transferred to a clean tube and the extract was dried under vacuum in a LabConco Centrivap Concentrator. The dried extracts were resuspended in dH_2O and filtered to remove particulate before analysis on reverse phase HPLC.

[0220] The reverse phase HPLC columns used for the analysis were either a Phenomenex C18 Aqua column (2 X 100 mm) or the Phenomenex C18 Aqua column (3 X 150 mm) used with the appropriate guard column. The HPLC was run at 0.2 ml/min (for the 2 X 100 mm column) or at 0.5 ml/min (for the 3 X 150 mm column) with the following buffer system: 5 mM tetrabutylammonium acetate, 50 mM ammonium phosphate, and an acetonitrile gradient running from 5% up to as high as 60%. The amount of remaining parent compound at each time point was used to determine the half-life of the compound. Time points were only taken through 48 hours so that if greater than 50% of a compound was still intact after 48 hours incubation the half-life was expressed as >48 hours. Unmodified nucleoside triphosphates were used as positive controls. Under these conditions unmodified nucleoside triphosphates had half-lives of approximately two hours.

Table 6.
Serum Half-Lives of NTPs and AZT Triphosphate Mimics

Compound	Half-Life (hours)
Adenosine Triphosphate	2
Thymidine Triphosphate	2
AZT Triphosphate	2
AZT α-*P*-Boranotriphosphate (Rp)	6
AZT α-*P*-Boranotriphosphate (Sp)	6
27–Isomer 1	>48
27–Isomer 2	>48
26–Isomer 1	45
26–Isomer 2	48
23	36

Table 7.
Serum Half-Lives of AZT Triphosphate Mimic Prodrugs

Compound Name	Half-Life (hours)
Adenosine Triphosphate	1.5
27-Isomer_	>48
69	>48
70	>48
42	>48

Table 8.
Serum Half-Lives of Gemcitibine Diphosphate Mimics and Their Prodrugs

Compound	Half-Life (hours)
Adenosine Triphosphate	2.5
6	15
75	>48
76	>48
5	>48

Example 51

Stability assessment using cell extracts

[0221] The stability of compounds in cell extracts was examined as follows:

[0222] Cell lysis buffer was added to cell pellets and the cells frozen and thawed 3X using dry ice. The lysis buffer (LB) was composed of the following: 50 mM Tris-HCl, pH 7.4 (100 μl/ml 10X stock), 20% glycerol (200 μl/ml), and 0.5% Triton X-100 (5μl/ml). 100 μl of LB was added to each microfuge tube containing 10^7 frozen CEM cells. After the cells were lysed the extracts were centrifuged at high speed in a microcentrifuge for 5 minutes and the clarified cell extract transferred to a new tube. The cell stability reaction mixtures contained concentrations of buffer, magnesium, nucleotide, and cell extract as shown below:

50 mM Tris-HCl, pH 7.4

0.1 mM MgCl2

500 μM nucleotide mimic (or control nucleotide)

50% cell extract (v/v)

[0223] The reaction mixtures were incubated at 37°C. At each time point, including at time zero, 12.5 μl aliquots were added to 40 μl ice-cold methanol. Typically, time points were taken after 30 and 60 minutes, and 2, 3, 8, 24, and 48 hours. The samples were incubated in methanol for at least 60 minutes and typically overnight at -20°C prior to further processing. The cell extracts were centrifuged at high speed in refrigerated microcentrifuge for 20 minutes and the supernatant transferred to a new tube. The extract was then dried under vacuum in a LabConco Centrivap Concentrator. The samples were then resuspended in 40 or 50 μl dH_2O, filtered to remove any particulate, and analyzed by reverse phase HPLC.

[0224] The reverse phase HPLC columns used for the analysis were either a Phenomenex C18 Aqua column (2 X 100 mm) or the Phenomenex C18 Aqua column (3 X 150 mm) used with the appropriate guard column. The HPLC flow rate was either 0.2 ml/min (for the 2 X 100 mm column) or 0.5 ml/min (for the 3 X 150 mm column) with the following buffer system: 5 mM tetrabutylammonium acetate, 50 mM ammonium phosphate, and an acetonitrile gradient running from 5% up to as high as 60%. The amount of remaining parent compound at each time point was used to determine the half-life of the compound. Time points were only taken through 48 hours so that if greater than 50% of a compound was still intact after 48 hours incubation the half-life was expressed as >48 hours. Unmodified nucleoside triphosphates were used as positive controls. Under these conditions unmodified nucleoside triphosphates had half-lives of approximately ten minutes.

Table 9.
Cell extract Half-Lives of AZT Triphosphate Mimics

Compound Number	Half-Life (hours)
Adenosine Triphosphate	.25
27–Isomer 1	>48
27–Isomer 2	>48
26–Isomer 1	>48
26–Isomer 2	>48

Table 10.
Cell Extract Half-Lives of AZT 5'-α-*P*-borano-β,γ-(difluoromethylene)triphosphate Prodrugs

Compound Number	Half-Life (hours)
Adenosine Triphosphate	.25
69	8
70	15
41	>48

Table 11.
Cell Extract Half-Lives of Gemcitibine 5'-Methylendiphosphonate and its Prodrugs

Compound Number	Half-Life (hours)
Adenosine Triphosphate	.25
75	19
76	37
5	>48

Example 52

Cell Uptake Procedure

[0225] CEM cells were resuspended in RPMI at 1 X 10^7 cells/mL. The appropriate amount of each compound was added to well and then 0.5 ml of the cells was aliquoted into the appropriate number of wells of a 24-well plate and incubated the cells at 37°C. Cells were harvested after 24 hours of incubation. To harvest cells they were transferred to a microfuge tube and spun for one minute at high speed. The samples were washed in PBS containing 1 mg/ml BSA and re-centrifuged. The PBS was then aspirated and 200 µL of ice-cold 60% methanol was added to extract the nucleotides. The extracts were incubated overnight at -20°C. The next day the samples were spun at high speed in a microfuge for 20 minutes and the supernatant transferred to clean tubes and dried in the speed vac. After drying the samples were resuspended in 50 µl, filtered, and the samples then directly injected onto the HPLC. The nucleotides were analyzed using reverse phase HPLC with ion-pairing agents for separation of the nucleotides. The procedure was adapted from the following paper: Furman, *et al., Proc. Natl. Acad. Sci.* USA **1986**, 83, 8333-8337.

Table 12.
Results from 24 hour incubation with AZTTP prodrug mimics

Compound Number	Incubation Concentration	Intracellular levels of 27 (pmol/10^6 cells)
	(μM)	
27-isomer 1	25	BLD
	50	5.6
	100	10.6
69	25	33.1
	50	51.1
	100	70.8
70	25	10.5
	50	15.6
	100	19.7

[0226] The limit of detection was estimated to be 5 pmol/10^6 cells.

Example 51

In vitro anti-HIV activity assay

[0227] The procedure was adapted from the following paper: Structure-Activity and Cross-Resistance Evaluations of a Series of Human Immunodeficiency Virus Type 1-Specific Compounds Related to Oxathiin Carboxanilide. Buckheit, Jr. *et al., Antimicrobial Agents and Chemotherapy*, **1995**, 39, 2718–2727.

CEM-SS/MTS Anti-HIV Assays

[0228] The antiviral efficacy of test compounds were evaluated in CEM-SS cell cultures infected with the lymphocyte-tropic virus strain HIV-1_{RF} at an MOI of approximately 0.01. At assay termination, assay plates were stained with the soluble tetrazolium-based dye MTS (CellTiter 96 Reagent, Promega) to determine cell viability and quantify compound toxicity. MTS is metabolized by the mitochondria enzymes of metabolically active cells to yield a soluble formazan product, allowing the rapid quantitative analysis of cell viability and compound cytotoxicity.

Efficacy Evaluation in CEM-SS Cells Anti-HIV-1 Cytoprotection Assay

[0229] Cell Preparation – CEM-SS cells were passaged in T-75 flasks prior to use in the antiviral assay. On the day preceding the assay, the cells were split 1:2 to assure they were in an exponential growth phase at the time of infection. Total cell and viability quantification was performed using a hemacytometer and trypan blue exclusion. Cell viability was greater than 95% for the cells to be utilized in the assay. The cells were resuspended at 5 x 10^4 cells/ml in tissue culture medium and added to the drug-containing microtiter plates in a volume of 50 µl.

[0230] Virus Preparation – The virus used for these tests was the lymphocytropic virus strain HIV-1_{RF}. This virus was obtained from the NIH AIDS Research and Reference Reagent Program and was grown in CEM-SS cells for the production of stock virus pools. For each assay, a pre-titered aliquot of virus was removed from the freezer (-80°C) and allowed to thaw slowly to room temperature in a biological safety cabinet. The virus was resuspended and diluted into tissue culture medium such that the amount of virus added to each well in a volume of 50 µl was the amount determined to give between 85 to 95% cell killing at 6 days post-infection. $TCID_{50}$ calculations by endpoint titration in CEM-SS cells indicated that the multiplicity of infection of these assays was approximately 0.01.

MTS staining for cell viability

[0231] At assay termination, the assay plates were stained with the soluble tetrazolium-based dye MTS (CellTiter Reagent Promega) to determine cell viability and quantify compound toxicity. MTS is metabolized by the mitochondrial enzymes of metabolically active cells to yield a soluble formazan product, allowing the rapid quantitative analysis of cell viability and compound cytotoxicity. The MTS is a stable solution that does not require preparation before use. At termination of the assay, 20 µL of MTS reagent was added per well. The microtiter plates were then incubated 4-6 hrs at 37°C for the HIV cytoprotection assay; the incubation intervals were chosen based on empirically determined times for optimal dye reduction. Adhesive plate sealers were used in place of the lids, the sealed plate was inverted several times to mix the soluble formazan product and the plate was read spectrophotometrically at 490 nm with a Molecular Devices Vmax plate reader.

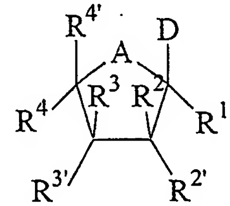
Table 13.
Anti-HIV Screening

Compound Number	CEM-SS IC_{50}	CEM-SS TC_{50}	Therapeutic Index
27-isomer 1	1.64 μM	> 200.0 μM	122.32
69	1.13 μM	45.9 μM	40.75
70	0.95 μM	> 200.0 μM	> 210.89
41	1.41 μM	12.0 μM	8.53
43	6.43 μM	125.0 μM	19.43
40	4.00 μM	> 200.0 μM	> 50.03
42	2.54 μM	32.1 μM	12.64

Claims

What is claimed:

1. A compound of Formula (I) which may be a D- or L-nucleotide:



(I)

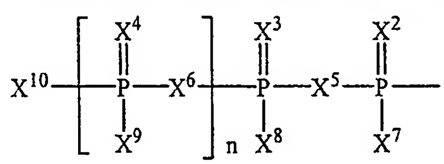
wherein

A is O, S, CY_2, NH or NR;

$R^{4'}$ is $-L-R^5$

L is selected from the group consisting of O, S, NH, NR, CY_2O, CY_2S, CY_2NH, CY_2, CY_2CY_2, CY_2OCY_2, CY_2SCY_2, and CY_2NHCY_2, wherein Y is selected from the group consisting of H, F, Cl, Br, alkyl, alkenyl, and alkynyl, wherein alkyl, alkenyl, and alkynyl may each optionally contain one or more heteroatoms;

R^5 is a di- or tri-phosphate moiety of Formula (II):



(II)

X^2, X^3, and X^4 are selected independently from the group consisting of O, S, Se, NH and NR;

X^5 and X^6 are selected independently from the group consisting of O, S, Se, O_2, CY_2CO, CHOH, $C(OH)_2$, CH_2O, CH_2CH_2, CH_2CHNH_2, $CH_2CH_2CHNH_2$, CY_2OCY_2, CY_2, CRY, CY_2CY_2, CHR, CC, HC=CH, NH, NR, NOH, NOR, NNH_2, and NNHR;

X^7, X^8, X^9, and X^{10} are selected independently from the group consisting of H, F, OH, SH, NH_2, NHOH, NHOR, $NHNH_2$, NHNHR, CN, N_3, $^{-}BH_3M^{+}$, R, OR, SR, SeH, SeR, NHR, NR_2, and R*, wherein R* is a prodrug substituent;

wherein at least one of X^7, X^8, X^9 and X^{10} is R*;

R* may be conjugated to one or more X^7-X^{10} positions

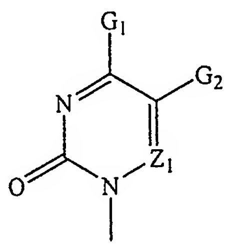
R is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, acyl, and aralkyl, each optionally containing one or more heteroatoms;

M^+ is a cation;

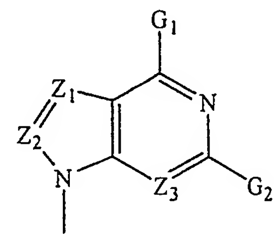
n is 0 or 1;

R^1, R^2, $R^{2'}$, R^3, $R^{3'}$, and R^4 are selected independently from the group consisting of H, F, Cl, Br, OH, SH, NH_2, NHOH, N_3, NO_2, CHO, COOH, CN, $CONH_2$, COOR, R, OR, SR, SSR, NHR, and NR_2; alternatively, R^2 and $R^{2'}$ together and R^3 and $R^{3'}$ together independently are =O, =S, or =J-Q, where J is N, CH, CF, CCl, or CBr and Q is H, F, Cl, Br, N_3, or R;

D is a nucleoside base of Formula (**III**) or (**IV**):



(**III**)

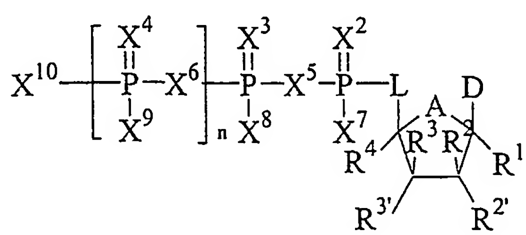


(**IV**)

Z^1, Z^2, and Z^3 are independently N, CH or C-G^3; and

G^1, G^2, and G^3 are selected independently from the group consisting of H, F, Cl, Br, I, OH, SH, NH_2, NHOH, $NHNH_2$, N_3, NO, NO_2, CHO, COOH, CN, $CONH_2$, CONHR, C(S)NH_2, C(S)NHR, COOR, R, OR, SR, NHR, and NR_2; when two of G^3 are present on a molecule they may be same as or different from each other.

2. The compound defined in claim 1 of Formula (**V**):



(**V**)

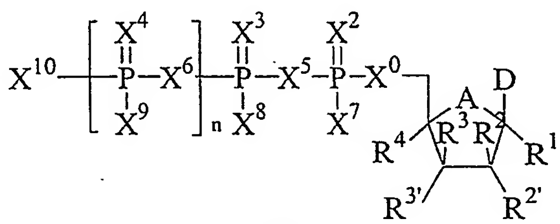
wherein L is selected from the group consisting of O, S, NH, CY_2, CY_2CY_2, and CH_2CY_2 where Y is H, F, Cl, or Br;

wherein X^2, X^3, and X^4 are O, S, Se ;

wherein X^5 and X^6 are selected independently from the group consisting of O, S, NH, NR, CY_2; and

wherein X^7, X^8, X^9, and X^{10} are selected independently from the group consisting of H, F, OH, SH, NH_2, NHOH, $^-BH_3M^+$, R, R*, OR, SR, and NHR.

3. The compound defined in claim 1 of Formula **(VI)**:



(VI)

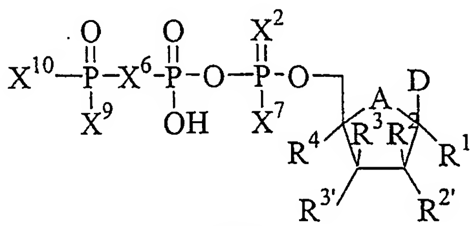
wherein X^0 is O, S, or NH;

wherein X^2, X^3, and X^4 are O or S;

wherein X^5 and X^6 are selected independently from the group consisting of O, S, NH, NR, and CY_2;

wherein X^7, X^8, X^9, and X^{10} are selected independently from the group consisting of H, F, OH, SH, NH_2, NHOH, $^-BH_3M^+$, R, R*, OR, SR, and NHR.

4. The compound defined in claim 1 of Formula **(VII)**:



(VII)

wherein X^2 is O or S;

wherein X^6 is NH or CY_2;

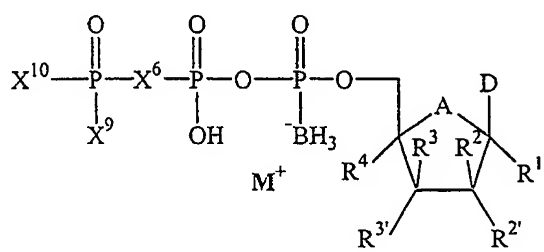
wherein X^7 is selected from the group consisting of H, F, SH, NH_2, NHOH, $^-BH_3M^+$, R, R*, SR, and NHR; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, NH_2, NHOH, $^-BH_3M^+$, R, R*, OR, SR, and NHR.

5. The compound defined in claim 4
wherein X^2 is O; and
wherein X^7 is SH, NH_2, $^-BH_3M^+$, alkyl, aryl, alkylamino, or arylamino.

6. The compound defined in claim 4
wherein X^2 is O; and
wherein X^7 is selected from the group consisting of SH, NH_2, $^-BH_3M^+$, R, and NHR.

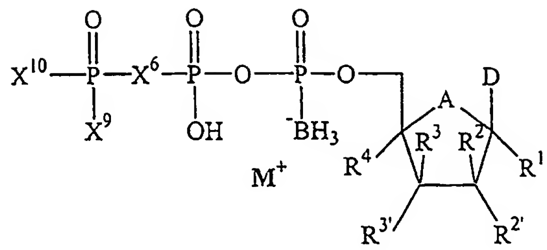
7. The compound defined in claim 1 of Formula **(VIII)**:



(VIII)

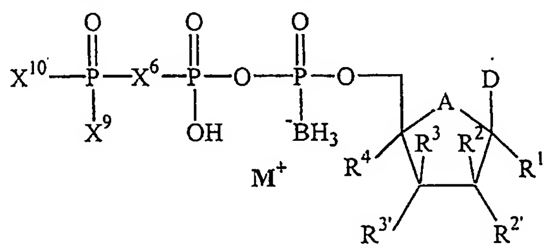
wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, alkyl, alkoxy, aryl, and aryloxy.

8. The compound defined in claim 1 of Formula (VIII):
wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceroxy, 1,2-*O*-dialkylglyceroxy, and 1-*O*-alkyl-2-*O*-acylglyceroxy.



(VIII)

9. The compound defined in claim 1 of Formula (VIII):



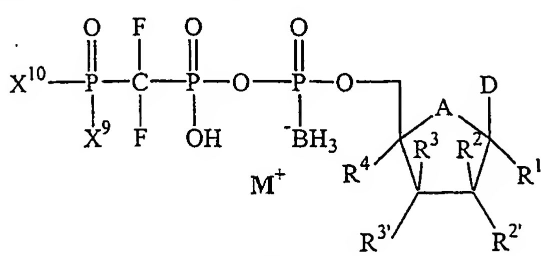
(VIII)

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy, acylthioethoxy, acyloxymethoxy, 1,2-O-diacylglyceryloxy, and 1-O-alkyl-2-O-acylglyceryloxy; and

wherein X^6 is selected from a group consisting of NH, CHF, CCl_2 and CF_2.

10. The compound defined in claim 8 wherein at least one of X^9 and X^{10} is 1,2-*O*-dialkylglyceryloxy.

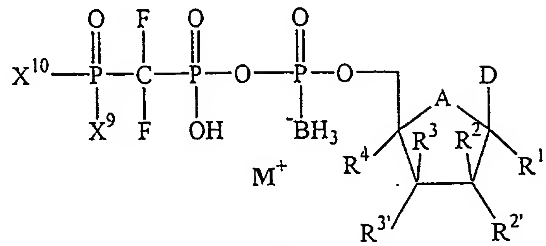
11. The compound defined in claim 1 of Formula **(IX)**:



(IX)

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy, and aryloxy.

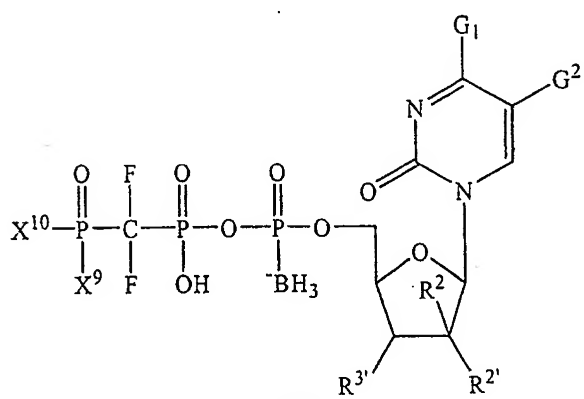
12. The compound defined in 1 of Formula (IX):



(IX)

wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

13. The compound defined in claim 1 of Formula (X):



(X)

wherein R^2 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

wherein $R^{2'}$ is H, F, OH, or OMe, alkyl, methoxyethoxy, or NH_2;

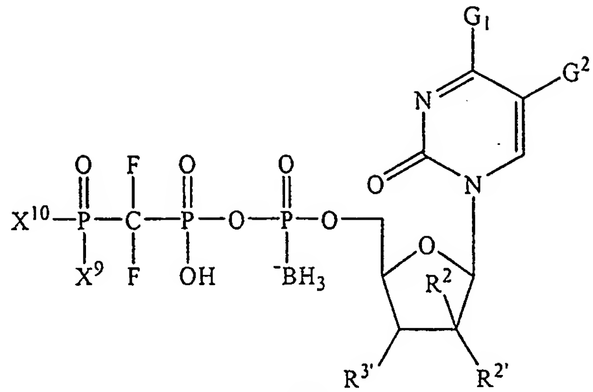
wherein $R^{3'}$ is H, F, OH, or N_3;

wherein G^1 is OH or NH_2;

wherein G^2 is H or Me;

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy, and aryloxy.

14. The compound defined in claim 1 of Formula **(X)**:



(X)

wherein $R^{2'}$ is H, F, OH, or OMe, alkyl, methoxyethoxy, or NH_2;

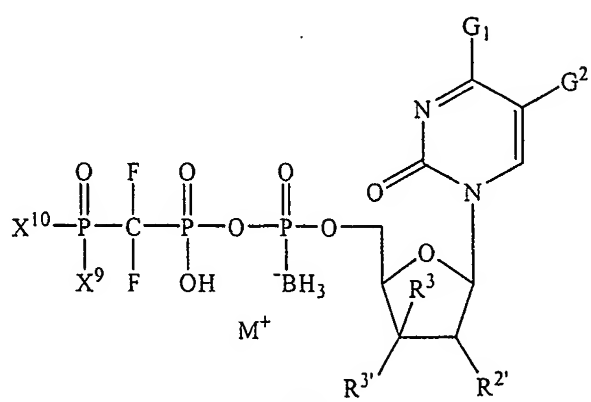
wherein $R^{3'}$ is H, F, OH, or N_3;

wherein G^1 is OH or NH_2;

wherein G^2 is H or Me;

wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

15. The compound defined in claim 1 of Formula **(XI)**:



(XI)

wherein $R^{2'}$ is H, F, OH, or OMe;

wherein R^3 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

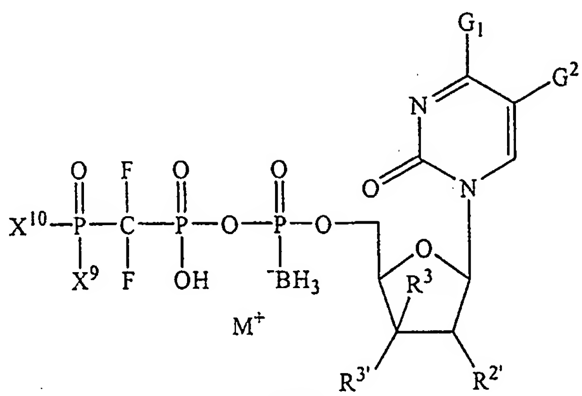
wherein $R^{3'}$ is H, F, OH, or N_3;

wherein G^1 is OH or NH_2;

wherein G^2 is H or Me; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy, and aryloxy.

16. The compound defined in claim 1 of Formula **(XI)**:



(XI)

wherein $R^{2'}$ is H, F, OH, or OMe;

wherein R^3 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

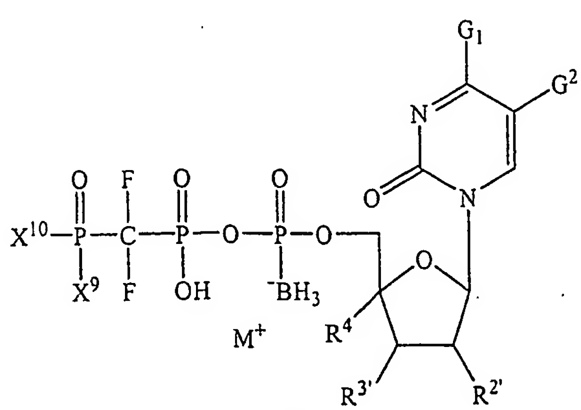
wherein $R^{3'}$ is H, F, OH, or N_3;

wherein G^1 is OH or NH_2;

wherein G^2 is H or Me; and

wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

17. The compound defined in claim 1 of Formula **(XII)**:



(XII)

wherein $R^{2'}$ is H, F, OH, or OMe;

wherein $R^{3'}$ is H, F, OH, or N_3;

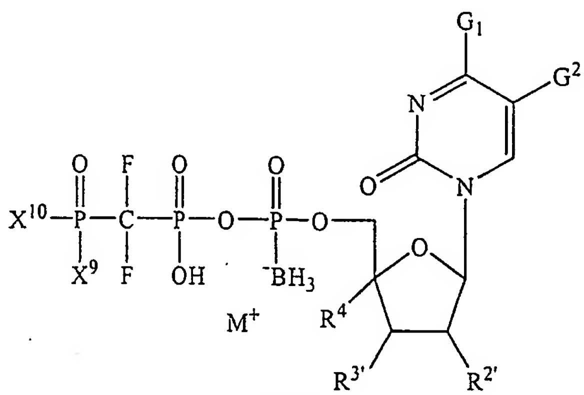
wherein R^4 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

wherein G^1 is OH or NH_2;

wherein G^2 is H or Me; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy, and aryloxy.

18. The compound defined in claim 1 of Formula **(XII)**:



(XII)

wherein $R^{2'}$ is H, F, OH, or OMe;

wherein $R^{3'}$ is H, F, OH, or N_3;

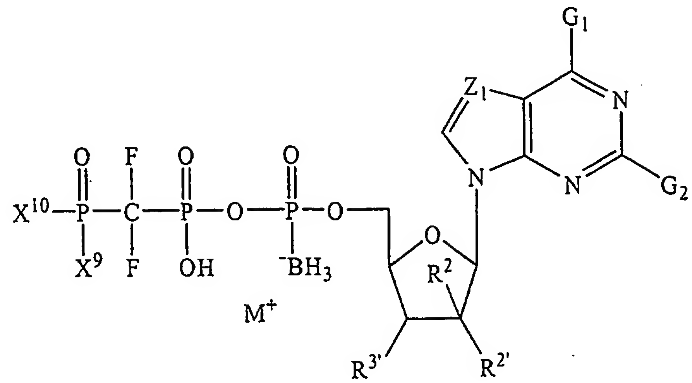
wherein R^4 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

wherein G^1 is OH or NH_2;

wherein G^2 is H or Me; and

wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

19. The compound defined in claim 1 of Formula **(XIII)**:



(XIII)

wherein R^2 is methyl, ethyl, vinyl, ethynyl, hydroxymethyl or haloalkyl;

wherein $R^{2'}$ is H, F, OH, OCH_3, or methoxyethoxy;

wherein $R^{3'}$ is H, F, OH, N_3, NH_2, or CH_2OH;

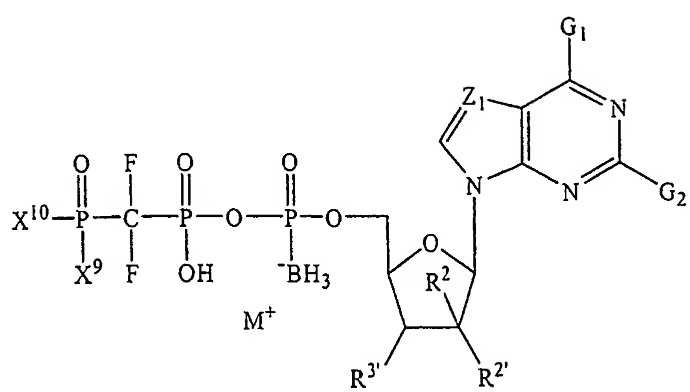
wherein G^1 is OH, NH_2, Cl, OMe, NH-cyclopropyl, SH, or S-alkyl;

wherein G^2 is H, NH_2, NHR, F, C1, Br, or I;

wherein Z^1 is N or CH; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy and aryloxy.

20. The compound defined in claim 1 of Formula **(XIII)**:



(XIII)

wherein R^2 is methyl, ethyl, vinyl, ethynyl, hydroxymethyl or haloalkyl;

wherein $R^{2'}$ is H, F, OH, OCH_3, or methoxyethoxy;

wherein $R^{3'}$ is H, F, OH, N_3, NH_2, or CH_2OH;

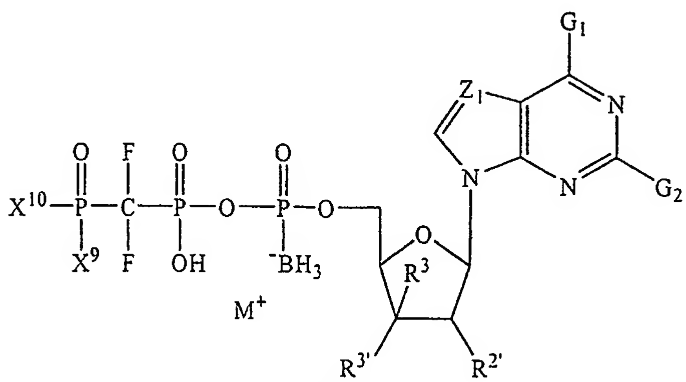
wherein G^1 is OH, NH_2, Cl, OMe, NH-cyclopropyl, SH, or S-alkyl;

wherein G^2 is H, NH_2, NHR, F, C1, Br, or I;

wherein Z^1 is N or CH; and

wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

21. The compound defined in claim 1 of Formula **(XIV)**:



(XIV)

wherein $R^{2'}$ is H, F, OH or OCH_3;

wherein R^3 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

wherein $R^{3'}$ is H, F, OH, or N_3;

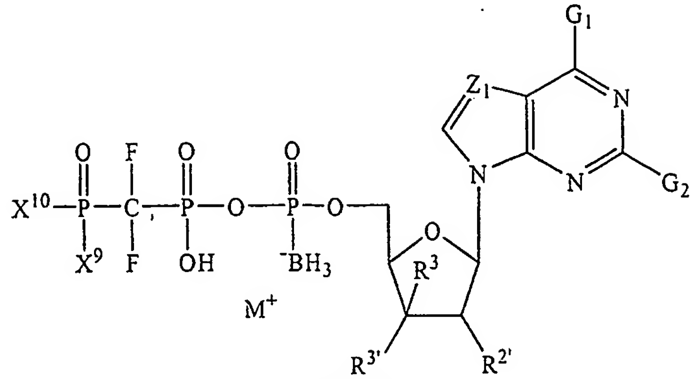
wherein G^1 is OH or NH_2;

wherein G^2 is H or NH_2;

wherein Z^1 is N or CH; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy and aryloxy.

22. The compound defined in claim 1 of Formula (XIV):



(XIV)

wherein $R^{2'}$ is H, F, OH or OCH_3;

wherein R^3 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

wherein $R^{3'}$ is H, F, OH, or N_3;

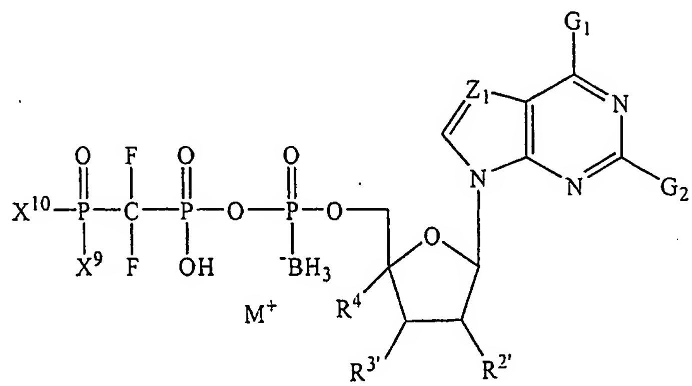
wherein G^1 is OH or NH_2;

wherein G^2 is H or NH_2;

wherein Z^1 is N or CH; and

wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

23. The compound defined in claim 1 of Formula (XV):



(XV)

wherein $R^{2'}$ is H, F, OH or OCH_3;

wherein $R^{3'}$ is H, F, OH, or N_3;

wherein R^4 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

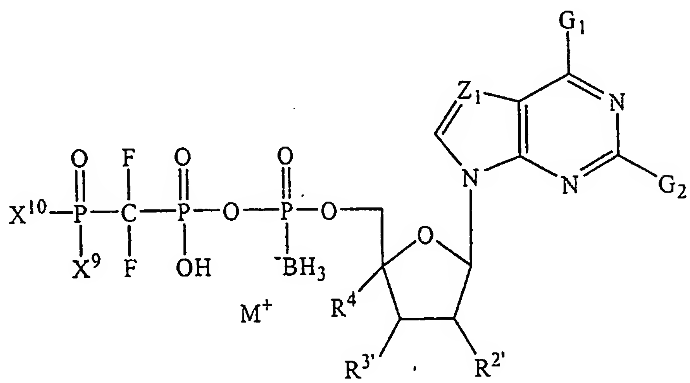
wherein G^1 is OH or NH_2;

wherein G^2 is H or NH_2;

wherein Z^1 is N or CH; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy and aryloxy.

24. The compound defined in claim 1 of Formula **(XV)**:



(XV)

wherein $R^{2'}$ is H, F, OH or OCH_3;

wherein $R^{3'}$ is H, F, OH, or N_3;

wherein R^4 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

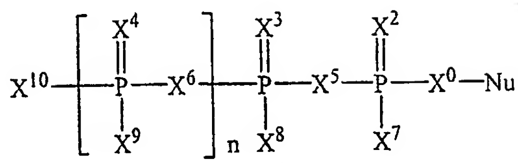
wherein G^1 is OH or NH_2;

wherein G^2 is H or NH_2;

wherein Z^1 is N or CH; and

wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

25. A compound of Formula **(XVI)**:



(XVI)

wherein X^0, X^5 and X^6 are selected independently from the group consisting of O, S, NH, and CY_2;

wherein Y is selected from the group consisting of H, F, Cl, Br, alkyl, alkenyl, and alkynyl, wherein alkyl, alkenyl, and alkynyl may each optionally contain one or more heteroatoms;

wherein X^2, X^3, and X^4 are selected independently from the group consisting of O, S, and Se;

wherein X^7, X^8, X^9, and X^{10} are selected independently from the group consisting of H, F, OH, SH, NH_2, NHOH, CN, N_3, $^-BH_3M^+$, R, R*, OR, SR, SeH, SeR, NHR, and NR_2;

wherein at least one of X^7, X^8, X^9 and X^{10} is R*;

wherein Nu is selected from the group consisting of natural nucleosides, sugar-modified nucleosides, base-modified nucleosides, and nucleosides with both sugar and base modifications;

wherein Nu is linked to X^0 through CH_2 of the sugar moiety of Nu; and

R is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, acyl, and aralkyl each optionally containing one or more heteroatoms;

with the proviso that one of X^7-X^{10} is selected from R*, wherein R* is a prodrug substituent.

26. The compound defined in claim 25 where Nu is selected from the group consisting of:

adenosine,
cytidine,
guanosine,
uridine,
2'-deoxyadenosine,
2'-deoxycytidine,

2'-deoxyguanosine,
thymidine,
inosine,

9-(β–D-arabinofuranosyl)adenine,
1-(β-D-arabinofuranosyl)cytosine,
9-(β-D-arabinofuranosyl)guanine,
1-(β-D-arabinofuranosyl)uracil,
9-(β–D-arabinofuranosyl)hypoxanthine,
1-(β-D-arabinofuranosyl)thymine,

3'-azido-3'-deoxythymidine,
3'-azido-2',3'-dideoxyuridine,
3'-azido-2',3'-dideoxycytidine,
3'-azido-2',3'-dideoxyadenosine,
3'-azido-2',3'-dideoxyguanosine,
3'-azido-2',3'-dideoxyinosine,

3'-deoxythymidine,
2',3'-dideoxyuridine,
2',3'-dideoxyinosine,
2',3'-dideoxyadenosine,
2',3'-dideoxycytidine,
2',3'-dideoxyguanosine,
9-(2,3-dideoxy-1-β-D-ribofuranosyl)-2,6-diaminopurine,

3'-deoxy-2',3'-didehydrothymidine,
2',3'-didehydro-2',3'-dideoxyuridine,
2',3'-didehydro-2',3'-dideoxycytidine,
2',3'-didehydro-2',3'-dideoxyadenosine,
2',3'-didehydro-2',3'-dideoxyguanosine,
2',3'-didehydro-2',3'-dideoxyinosine,

3-deazaadenosine,
3-deazaguanosine,
3-deazainosine,
7-deazaadenosine,
7-deazaguanosine,
7-deazainosine,

6-azauridine,
6-azathymidine,
6-azacytidine,
5-azacytidine,

9-(β-D-ribofuranosyl)-6-thiopurine,
6-methylthio-9-(β-D-ribofuranosyl)purine,

2-amino-9-(β-D-ribofuranosyl)-6-thiopurine,
2-amino-6-methylthio-9-(β-D-ribofuranosyl)purine,

5-fluorocytidine,
5-iodocytidine,
5-bromocytidine,
5-chlorocytidine,
5-fluorouridine,
5-iodouridine,
5-bromouridine,
5-chlorouridine,

2'-*C*-methyladenosine,
2'-*C*-methylcytidine,
2'-*C*-methylguanosine,
2'-*C*-methylinosine,
2'-*C*-methyluridine,
2'-*C*-methylthymidine,

2'-deoxy-2'-fluoroadenosine,
2'-deoxy-2'-fluorocytidine,
2'-deoxy-2'-fluoroguanosine,
2'-deoxy-2'-fluorouridine,
2'-deoxy-2'-fluoroinosine,
2'-α-fluorothymidine,

2'-deoxy-2'-fluoroarabinoadenosine,
2'-deoxy-2'-fluoroarabinocytidine,
2'-deoxy-2'-fluoroarabinoguanosine,
2'-deoxy-2'-fluoroarabinouridine,
2'-deoxy-2'-fluoroarabinoinosine,
2'-β-fluorothymidine,

2'-*O*-methyladenosine,
2'-*O*-methylcytidine,
2'-*O*-methylguanosine,
2'-*O*-methylinosine,
2'-*O*-5-dimethyluridine,

2'-*C*-ethynylcytidine,
2'-*C*-ethynylguanosine,
2'-*C*-ethynyluridine,
2'-*C*-ethynylinosine,
2'-*C*-ethynyl-5-methyluridine,

3'-*C*-ethynyladenosine,
3'-*C*-ethynylcytidine,

3'-*C*-ethynylguanosine,
3'-*C*-ethynyluridine,
3'-*C*-ethynylinosine,
3'-*C*-ethynyl-5-methyluridine,

3'-deoxyadenosine,
3'-deoxycytidine,
3'-deoxyguanosine,
3'-deoxyuridine,
3'-deoxyinosine,

4'-*C*-ethynyladenosine,
4'-*C*-ethynylcytidine,
4'-*C*-ethynylguanosine,
4'-*C*-ethynyluridine,
4'-*C*-ethynylinosine,
4'-*C*-ethynylthymidine,

4'-*C*-methyladenosine,
4'-*C*-methylcytidine,
4'-*C*-methylguanosine,
4'-*C*-methyluridine,
4'-*C*-methylinosine,
4'-*C*-methylthymidine,

2'-*C*-methyl-7-deazaadenosine,
2'-*C*-methyl-7-deazaguanosine,
2'-*C*-methyl-3-deazaadenosine,
2'-*C*-methyl-3-deazaguanosine,
2'-*O*-methyl-7-deazaadenosine,
2'-*O*-methyl-7-deazaguanosine,
2'-*O*-methyl-3-deazaadenosine,
2'-*O*-methyl-3-deazaguanosine,
2'-*C*-methyl-6-azauridine,
2'-*C*-methyl-5-fluorouridine,
2'-*C*-methyl-5-fluorocytidine,
2'-*C*-methyl-2-chloroadenosine,

2'-deoxy-7-deazaadenosine,
2'-deoxy-3-deazaadenosine,
2'-deoxy-7-deazaguanosine,
2'-deoxy-3-deazaguanosine,
2'-deoxy-6-azauridine,
2'-deoxy-5-fluorouridine,
2'-deoxy-5-fluorocytidine,
2'-deoxy-5-iodouridine,
2'-deoxy-5-iodocytidine,

2'-deoxy-2-chloroadenosine,
2'-deoxy-2-fluoroadenosine,

3'-deoxy-7-deazaadenosine,
3'-deoxy-7-deazaguanosine,
3'-deoxy-3-deazaadenosine,
3'-deoxy-3-deazaguanosine,
3'-deoxy-6-azauridine,
3'-deoxy-5-fluorouridine,
3'-deoxy-5-iodouridine,
3'-deoxy-5-fluorocytidine,
3'-deoxy-2-chloroadenosine,

2',3'-dideoxy-7-deazaadenosine,
2',3'-dideoxy-7-deazaguanosine,
2',3'-dideoxy-3-deazaadenosine,
2',3'-dideoxy-3-deazaguanosine,
2',3'-dideoxy-6-azauridine,
2',3'-dideoxy-5-fluorouridine,
2',3'-dideoxy-5-fluorouridine,
2',3'-dideoxy-5-iodocytidine,
2',3'-dideoxy-2-chloroadenosine,

2',3'-dideoxy-β-L-cytidine,
2',3'-dideoxy-β-L-adenosine,
2',3'-dideoxy-β-L-guanosine,
3'-deoxy-β-L-thymidine,
2',3'-dideoxy-5-fluoro-β-L-cytidine,

β-L-thymidine,
2'-deoxy-β-L-cytidine,
2'-deoxy-β-L-adenosine,
2'-deoxy-β-L-guanosine,
2'-deoxy-β-L-inosine,

β-L-cytidine,
β-L-adenosine,
β-L-guanosine,
β-L-uridine,
β-L-inosine,

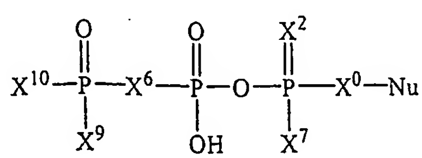
2',3'-didehydro-2',3'-dideoxy-β-L-cytidine,
2',3'-didehydro-3'-dideoxy-β-L-thymidine,
2',3'-didehydro-2',3'-dideoxy-β-L-adenosine,
2',3'-didehydro-2',3'-dideoxy-β-L-guanosine,
2',3'-didehydro-2',3'-dideoxy-β-L-5-fluorocytidine,

2'-deoxy-2',2'-difluorocytidine,
9-(β-D-arabinofuranosyl)-2-fluoroadenine,
2'-deoxy-2'(E)-fluoromethylenecytidine,
2'-deoxy-2'(Z)-fluoromethylenecytidine,
(-)-2',3'-dideoxy-3'-thiacytidine,
(+)-2',3'-dideoxy-3'-thiacytidine,

1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-D-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-L-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-D-ribofuranosyl-5-ethynylimidazole-4-carboxamide,
1-β-L-ribofuranosyl-5-ethynylimidazole-4-carboxamide,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine,
1-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)-5-methyluracil,
1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil,
E-5-(2-bromovinyl)-2'-deoxyuridine,
5-trifluoromethylthymidine,
1-β-D-arabinofuranosyl-5-propynyluracil,
1-(2-deoxy-2-fluoro-1-β-D-arabinofuranosyl)-5-ethyluracil,
2',3'-dideoxy-3'-fluoroguanosine,
3'-deoxy-3'-fluorothymidine,

(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(1*R*,3*S*,4*R*)-9-(3-hydroxy-4-hydroxymethylcyclopent-1-yl)guanine,
(1*S*,2*R*,4*R*)-9-(1-hydroxy-2-hydroxymethylcyclopent-4-yl)guanine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)-2,6-diaminopurine,
(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)cytosine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)guanine,
(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)-5-fluorocytosine,
(1*R*,2*S*,4*S*)-9-(4-hydroxy-3-hydroxymethyl-2-methylenecyclopent-4-yl]guanine, and
(1*S*,3*R*,4*S*)-9-(3-hydroxy-4-hydroxymethyl-5-methylenecyclopent-1-yl]guanine.

27. The compound defined in claim 25 of Formula **(XVII)**:



(XVII)

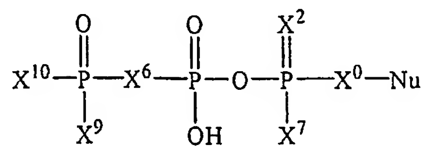
wherein X^0 and X^6 are selected independently from the group consisting of O, S, NH, CH_2, CHCl, CHBr, CHF,. CCl_2, CBr_2, and CF_2

wherein X^2 is O or S;

wherein X^7 is selected from the group consisting of OH, F, SH, NH_2, NHOH, $^-BH_3M^+$, R, SR, and NHR; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, NH_2, NHOH, $^-BH_3M^+$, R, R*, OR, SR and NHR.

28. The compound defined in claim 25 of Formula **(XVII)**:



(XVII)

wherein X^0 and X^6 are selected independently from the group consisting of O, S, NH, CH_2, CHCl, CHBr, CHF, CCl_2, CBr_2, and CF_2

wherein X^2 is O or S;

wherein X^7 is selected from the group consisting of OH, F, SH, NH_2, NHOH $^-BH_3M^+$, R, SR, and NHR; and

wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

29. The compound defined in claim 27 where Nu is selected from the group consisting of:

adenosine,
cytidine,
guanosine,
uridine,
2'-deoxyadenosine,
2'-deoxycytidine,
2'-deoxyguanosine,
thymidine,
inosine,

9-(β–D-arabinofuranosyl)adenine,
1-(β-D-arabinofuranosyl)cytosine,
9-(β-D-arabinofuranosyl)guanine,
1-(β-D-arabinofuranosyl)uracil,
9-(β–D-arabinofuranosyl)hypoxanthine,
1-(β-D-arabinofuranosyl)thymine,

3'-azido-3'-deoxythymidine,
3'-azido-2',3'-dideoxyuridine,
3'-azido-2',3'-dideoxycytidine,
3'-azido-2',3'-dideoxyadenosine,
3'-azido-2',3'-dideoxyguanosine,
3'-azido-2',3'-dideoxyinosine,

3'-deoxythymidine,
2',3'-dideoxyuridine,
2',3'-dideoxyinosine,
2',3'-dideoxyadenosine,
2',3'-dideoxycytidine,
2',3'-dideoxyguanosine,
9-(2,3-dideoxy-1-β-D-ribofuranosyl)-2,6-diaminopurine,

3'-deoxy-2',3'-didehydrothymidine,
2',3'-didehydro-2',3'-dideoxyuridine,
2',3'-didehydro-2',3'-dideoxycytidine,
2',3'-didehydro-2',3'-dideoxyadenosine,
2',3'-didehydro-2',3'-dideoxyguanosine,
2',3'-didehydro-2',3'-dideoxyinosine,

3-deazaadenosine,
3-deazaguanosine,
3-deazainosine,
7-deazaadenosine,
7-deazaguanosine,
7-deazainosine,

6-azauridine,
6-azathymidine,
6-azacytidine,
5-azacytidine,

9-(β-D-ribofuranosyl)-6-thiopurine,
6-methylthio-9-(β-D-ribofuranosyl)purine,
2-amino-9-(β-D-ribofuranosyl)-6-thiopurine,
2-amino-6-methylthio-9-(β-D-ribofuranosyl)purine,

5-fluorocytidine,

5-iodocytidine,
5-bromocytidine,
5-chlorocytidine,
5-fluorouridine,
5-iodouridine,
5-bromouridine,
5-chlorouridine,

2'-*C*-methyladenosine,
2'-*C*-methylcytidine,
2'-*C*-methylguanosine,
2'-*C*-methylinosine,
2'-*C*-methyluridine,
2'-*C*-methylthymidine,

2'-deoxy-2'-fluoroadenosine,
2'-deoxy-2'-fluorocytidine,
2'-deoxy-2'-fluoroguanosine,
2'-deoxy-2'-fluorouridine,
2'-deoxy-2'-fluoroinosine,
2'-α-fluorothymidine,

2'-deoxy-2'-fluoroarabinoadenosine,
2'-deoxy-2'-fluoroarabinocytidine,
2'-deoxy-2'-fluoroarabinoguanosine,
2'-deoxy-2'-fluoroarabinouridine,
2'-deoxy-2'-fluoroarabinoinosine,
2'-β-fluorothymidine,

2'-*O*-methyladenosine,
2'-*O*-methylcytidine,
2'-*O*-methylguanosine,
2'-*O*-methylinosine,
2'-*O*-5-dimethyluridine,

2'-*C*-ethynylcytidine,
2'-*C*-ethynylguanosine,
2'-*C*-ethynyluridine,
2'-*C*-ethynylinosine,
2'-*C*-ethynyl-5-methyluridine,

3'-*C*-ethynyladenosine,
3'-*C*-ethynylcytidine,
3'-*C*-ethynylguanosine,
3'-*C*-ethynyluridine,
3'-*C*-ethynylinosine,
3'-*C*-ethynyl-5-methyluridine,

3'-deoxyadenosine,
3'-deoxycytidine,
3'-deoxyguanosine,
3'-deoxyuridine,
3'-deoxyinosine,

4'-*C*-ethynyladenosine,
4'-*C*-ethynylcytidine,
4'-*C*-ethynylguanosine,
4'-*C*-ethynyluridine,
4'-*C*-ethynylinosine,
4'-*C*-ethynylthymidine,

4'-*C*-methyladenosine,
4'-*C*-methylcytidine,
4'-*C*-methylguanosine,
4'-*C*-methyluridine,
4'-*C*-methylinosine,
4'-*C*-methylthymidine,

2'-*C*-methyl-7-deazaadenosine,
2'-*C*-methyl-7-deazaguanosine,
2'-*C*-methyl-3-deazaadenosine,
2'-*C*-methyl-3-deazaguanosine,
2'-*O*-methyl-7-deazaadenosine,
2'-*O*-methyl-7-deazaguanosine,
2'-*O*-methyl-3-deazaadenosine,
2'-*O*-methyl-3-deazaguanosine,
2'-*C*-methyl-6-azauridine,
2'-*C*-methyl-5-fluorouridine,
2'-*C*-methyl-5-fluorocytidine,
2'-*C*-methyl-2-chloroadenosine,

2'-deoxy-7-deazaadenosine,
2'-deoxy-3-deazaadenosine,
2'-deoxy-7-deazaguanosine,
2'-deoxy-3-deazaguanosine,
2'-deoxy-6-azauridine,
2'-deoxy-5-fluorouridine,
2'-deoxy-5-fluorocytidine,
2'-deoxy-5-iodouridine,
2'-deoxy-5-iodocytidine,
2'-deoxy-2-chloroadenosine,
2'-deoxy-2-fluoroadenosine,

3'-deoxy-7-deazaadenosine,

3'-deoxy-7-deazaguanosine,
3'-deoxy-3-deazaadenosine,
3'-deoxy-3-deazaguanosine,
3'-deoxy-6-azauridine,
3'-deoxy-5-fluorouridine,
3'-deoxy-5-iodouridine,
3'-deoxy-5-fluorocytidine,
3'-deoxy-2-chloroadenosine,

2',3'-dideoxy-7-deazaadenosine,
2',3'-dideoxy-7-deazaguanosine,
2',3'-dideoxy-3-deazaadenosine,
2',3'-dideoxy-3-deazaguanosine,
2',3'-dideoxy-6-azauridine,
2',3'-dideoxy-5-fluorouridine,
2',3'-dideoxy-5-fluorouridine,
2',3'-dideoxy-5-iodocytidine,
2',3'-dideoxy-2-chloroadenosine,

2',3'-dideoxy-β-L-cytidine,
2',3'-dideoxy-β-L-adenosine,
2',3'-dideoxy-β-L-guanosine,
3'-deoxy-β-L-thymidine,
2',3'-dideoxy-5-fluoro-β-L-cytidine,

β-L-thymidine,
2'-deoxy-β-L-cytidine,
2'-deoxy-β-L-adenosine,
2'-deoxy-β-L-guanosine,
2'-deoxy-β-L-inosine,

β-L-cytidine,
β-L-adenosine,
β-L-guanosine,
β-L-uridine,
β-L-inosine,

2',3'-didehydro-2',3'-dideoxy-β-L-cytidine,
2',3'-didehydro-3'-dideoxy-β-L-thymidine,
2',3'-didehydro-2',3'-dideoxy-β-L-adenosine,
2',3'-didehydro-2',3'-dideoxy-β-L-guanosine,
2',3'-didehydro-2',3'-dideoxy-β-L-5-fluorocytidine,

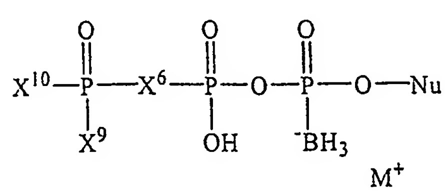
2'-deoxy-2',2'-difluorocytidine,
9-(β-D-arabinofuranosyl)-2-fluoroadenine,
2'-deoxy-2'(E)-fluoromethylenecytidine,

2'-deoxy-2'(Z)-fluoromethylenecytidine,
(-)-2',3'-dideoxy-3'-thiacytidine,
(+)-2',3'-dideoxy-3'-thiacytidine,

1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-D-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-L-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-D-ribofuranosyl-5-ethynylimidazole-4-carboxamide,
1-β-L-ribofuranosyl-5-ethynylimidazole-4-carboxamide,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine,
1-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)-5-methyluracil,
1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil,
E-5-(2-bromovinyl)-2'-deoxyuridine,
5-trifluoromethylthymidine,
1-β-D-arabinofuranosyl-5-propynyluracil,
1-(2-deoxy-2-fluoro-1-β-D-arabinofuranosyl)-5-ethyluracil,
2',3'-dideoxy-3'-fluoroguanosine,
3'-deoxy-3'-fluorothymidine,

(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(1*R*,3*S*,4*R*)-9-(3-hydroxy-4-hydroxymethylcyclopent-1-yl)guanine,
(1*S*,2*R*,4*R*)-9-(1-hydroxy-2-hydroxymethylcyclopent-4-yl)guanine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)-2,6-diaminopurine,
(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)cytosine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)guanine,
(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)-5-fluorocytosine,
(1*R*,2*S*,4*S*)-9-(4-hydroxy-3-hydroxymethyl-2-methylenecyclopent-4-yl]guanine, and
(1*S*,3*R*,4*S*)-9-(3-hydroxy-4-hydroxymethyl-5-methylenecyclopent-1-yl]guanine.

30. The compound defined in claim 25 of Formula **(XVIII)**:

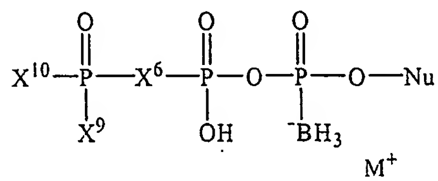


(XVIII)

wherein X^6 is selected from the group consisting of NH, CH_2, CHCl, CHBr, CHF, CCl_2, CBr_2, and CF_2; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, alkyl, alkoxy, aryl and aryloxy.

31. The compound defined in claim 25 of Formula **(XVIII)**:



(XVIII)

wherein X^6 is selected from the group consisting of NH, CH_2, CHCl, CHBr, CHF, CCl_2, CBr_2, and CF_2; and

wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceroxy, 1,2-*O*-dialkylglyceroxy, and 1-*O*-alkyl-2-*O*-acylglyceroxy.

32. The compound defined in claim 31 where Nu is selected from the group consisting of:

adenosine,
cytidine,
guanosine,
uridine,
2'-deoxyadenosine,
2'-deoxycytidine,
2'-deoxyguanosine,
thymidine,
inosine,

9-(β–D-arabinofuranosyl)adenine,
1-(β-D-arabinofuranosyl)cytosine,
9-(β-D-arabinofuranosyl)guanine,
1-(β-D-arabinofuranosyl)uracil,
9-(β–D-arabinofuranosyl)hypoxanthine,
1-(β-D-arabinofuranosyl)thymine,

3'-azido-3'-deoxythymidine,
3'-azido-2',3'-dideoxyuridine,

3'-azido-2',3'-dideoxycytidine,
3'-azido-2',3'-dideoxyadenosine,
3'-azido-2',3'-dideoxyguanosine,
3'-azido-2',3'-dideoxyinosine,

3'-deoxythymidine,
2',3'-dideoxyuridine,
2',3'-dideoxyinosine,
2',3'-dideoxyadenosine,
2',3'-dideoxycytidine,
2',3'-dideoxyguanosine,
9-(2,3-dideoxy-1-β-D-ribofuranosyl)-2,6-diaminopurine,

3'-deoxy-2',3'-didehydrothymidine,
2',3'-didehydro-2',3'-dideoxyuridine,
2',3'-didehydro-2',3'-dideoxycytidine,
2',3'-didehydro-2',3'-dideoxyadenosine,
2',3'-didehydro-2',3'-dideoxyguanosine,
2',3'-didehydro-2',3'-dideoxyinosine,

3-deazaadenosine,
3-deazaguanosine,
3-deazainosine,
7-deazaadenosine,
7-deazaguanosine,
7-deazainosine,

6-azauridine,
6-azathymidine,
6-azacytidine,
5-azacytidine,

9-(β-D-ribofuranosyl)-6-thiopurine,
6-methylthio-9-(β-D-ribofuranosyl)purine,
2-amino-9-(β-D-ribofuranosyl)-6-thiopurine,
2-amino-6-methylthio-9-(β-D-ribofuranosyl)purine,

5-fluorocytidine,
5-iodocytidine,
5-bromocytidine,
5-chlorocytidine,
5-fluorouridine,
5-iodouridine,
5-bromouridine,
5-chlorouridine,

2'-*C*-methyladenosine,

2'-*C*-methylcytidine,
2'-*C*-methylguanosine,
2'-*C*-methylinosine,
2'-*C*-methyluridine,
2'-*C*-methylthymidine,

2'-deoxy-2'-fluoroadenosine,
2'-deoxy-2'-fluorocytidine,
2'-deoxy-2'-fluoroguanosine,
2'-deoxy-2'-fluorouridine,
2'-deoxy-2'-fluoroinosine,
2'-α-fluorothymidine,

2'-deoxy-2'-fluoroarabinoadenosine,
2'-deoxy-2'-fluoroarabinocytidine,
2'-deoxy-2'-fluoroarabinoguanosine,
2'-deoxy-2'-fluoroarabinouridine,
2'-deoxy-2'-fluoroarabinoinosine,
2'-β-fluorothymidine,

2'-*O*-methyladenosine,
2'-*O*-methylcytidine,
2'-*O*-methylguanosine,
2'-*O*-methylinosine,
2'-*O*-5-dimethyluridine,

2'-*C*-ethynylcytidine,
2'-*C*-ethynylguanosine,
2'-*C*-ethynyluridine,
2'-*C*-ethynylinosine,
2'-*C*-ethynyl-5-methyluridine,

3'-*C*-ethynyladenosine,
3'-*C*-ethynylcytidine,
3'-*C*-ethynylguanosine,
3'-*C*-ethynyluridine,
3'-*C*-ethynylinosine,
3'-*C*-ethynyl-5-methyluridine,

3'-deoxyadenosine,
3'-deoxycytidine,
3'-deoxyguanosine,
3'-deoxyuridine,
3'-deoxyinosine,

4'-*C*-ethynyladenosine,
4'-*C*-ethynylcytidine,

4'-*C*-ethynylguanosine,
4'-*C*-ethynyluridine,
4'-*C*-ethynylinosine,
4'-*C*-ethynylthymidine,

4'-*C*-methyladenosine,
4'-*C*-methylcytidine,
4'-*C*-methylguanosine,
4'-*C*-methyluridine,
4'-*C*-methylinosine,
4'-*C*-methylthymidine,

2'-*C*-methyl-7-deazaadenosine,
2'-*C*-methyl-7-deazaguanosine,
2'-*C*-methyl-3-deazaadenosine,
2'-*C*-methyl-3-deazaguanosine,
2'-*O*-methyl-7-deazaadenosine,
2'-*O*-methyl-7-deazaguanosine,
2'-*O*-methyl-3-deazaadenosine,
2'-*O*-methyl-3-deazaguanosine,
2'-*C*-methyl-6-azauridine,
2'-*C*-methyl-5-fluorouridine,
2'-*C*-methyl-5-fluorocytidine,
2'-*C*-methyl-2-chloroadenosine,

2'-deoxy-7-deazaadenosine,
2'-deoxy-3-deazaadenosine,
2'-deoxy-7-deazaguanosine,
2'-deoxy-3-deazaguanosine,
2'-deoxy-6-azauridine,
2'-deoxy-5-fluorouridine,
2'-deoxy-5-fluorocytidine,
2'-deoxy-5-iodouridine,
2'-deoxy-5-iodocytidine,
2'-deoxy-2-chloroadenosine,
2'-deoxy-2-fluoroadenosine,

3'-deoxy-7-deazaadenosine,
3'-deoxy-7-deazaguanosine,
3'-deoxy-3-deazaadenosine,
3'-deoxy-3-deazaguanosine,
3'-deoxy-6-azauridine,
3'-deoxy-5-fluorouridine,
3'-deoxy-5-iodouridine,
3'-deoxy-5-fluorocytidine,
3'-deoxy-2-chloroadenosine,

2',3'-dideoxy-7-deazaadenosine,
2',3'-dideoxy-7-deazaguanosine,
2',3'-dideoxy-3-deazaadenosine,
2',3'-dideoxy-3-deazaguanosine,
2',3'-dideoxy-6-azauridine,
2',3'-dideoxy-5-fluorouridine,
2',3'-dideoxy-5-fluorouridine,
2',3'-dideoxy-5-iodocytidine,
2',3'-dideoxy-2-chloroadenosine,

2',3'-dideoxy-β-L-cytidine,
2',3'-dideoxy-β-L-adenosine,
2',3'-dideoxy-β-L-guanosine,
3'-deoxy-β-L-thymidine,
2',3'-dideoxy-5-fluoro-β-L-cytidine,

β-L-thymidine,
2'-deoxy-β-L-cytidine,
2'-deoxy-β-L-adenosine,
2'-deoxy-β-L-guanosine,
2'-deoxy-β-L-inosine,

β-L-cytidine,
β-L-adenosine,
β-L-guanosine,
β-L-uridine,
β-L-inosine,

2',3'-didehydro-2',3'-dideoxy-β-L-cytidine,
2',3'-didehydro-3'-dideoxy-β-L-thymidine,
2',3'-didehydro-2',3'-dideoxy-β-L-adenosine,
2',3'-didehydro-2',3'-dideoxy-β-L-guanosine,
2',3'-didehydro-2',3'-dideoxy-β-L-5-fluorocytidine,

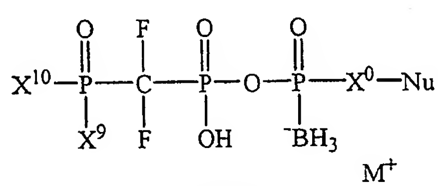
2'-deoxy-2',2'-difluorocytidine,
9-(β-D-arabinofuranosyl)-2-fluoroadenine,
2'-deoxy-2'(E)-fluoromethylenecytidine,
2'-deoxy-2'(Z)-fluoromethylenecytidine,
(-)-2',3'-dideoxy-3'-thiacytidine,
(+)-2',3'-dideoxy-3'-thiacytidine,

1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-D-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-L-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-D-ribofuranosyl-5-ethynylimidazole-4-carboxamide,

1-β-L-ribofuranosyl-5-ethynylimidazole-4-carboxamide,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine,
1-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)-5-methyluracil,
1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil,
E-5-(2-bromovinyl)-2'-deoxyuridine,
5-trifluoromethylthymidine,
1-β-D-arabinofuranosyl-5-propynyluracil,
1-(2-deoxy-2-fluoro-1-β-D-arabinofuranosyl)-5-ethyluracil,
2',3'-dideoxy-3'-fluoroguanosine,
3'-deoxy-3'-fluorothymidine,

(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(1*R*,3*S*,4*R*)-9-(3-hydroxy-4-hydroxymethylcyclopent-1-yl)guanine,
(1*S*,2*R*,4*R*)-9-(1-hydroxy-2-hydroxymethylcyclopent-4-yl)guanine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)-2,6-diaminopurine,
(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)cytosine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)guanine,
(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)-5-fluorocytosine,
(1*R*,2*S*,4*S*)-9-(4-hydroxy-3-hydroxymethyl-2-methylenecyclopent-4-yl]guanine, and
(1*S*,3*R*,4*S*)-9-(3-hydroxy-4-hydroxymethyl-5-methylenecyclopent-1-yl]guanine.

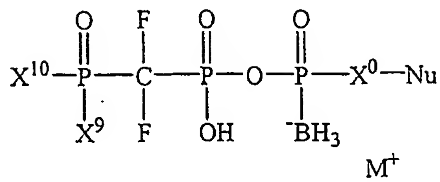
33. The compound defined in claim 25 of Formula **(XIX)**:



(XIX)

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, alkyl, alkoxy, aryl and aryloxy.

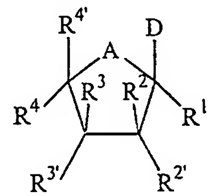
34. The compound defined in claim 25 of Formula **(XIX)**:



(XIX)

wherein X^9 and X^{10} are selected independently from the group consisting of acylthioethoxy, acyloxymethoxy, 1,2-*O*-diacylglyceryloxy, 1,2-*O*-dialkylglyceryloxy, and 1-*O*-alkyl-2-*O*-acylglyceryloxy.

35. A compound of Formula **(I)** which may be a D- or L-nucleotide:



(I)

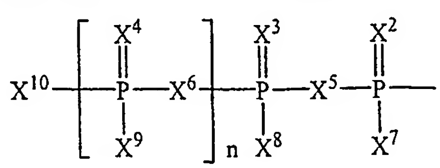
wherein

A is O, S, CY_2, NH or NR;

$R^{4'}$ is $-L-R^5$

L is selected from the group consisting of O, S, NH, NR, CY_2O, CY_2S, CY_2NH, CY_2, CY_2CY_2, CY_2OCY_2, CY_2SCY_2, and CY_2NHCY_2, CY_2SCY_2, and wherein Y is selected from the group consisting of H, F, Cl, Br, alkyl, alkenyl, and alkynyl, wherein alkyl, alkenyl, and alkynyl optionally contain one or more heteroatoms;

R^5 is a di- or tri-phosphate moiety of Formula **(II)**:



(II)

N is O or 1;

X^2, X^3, and X^4 are selected independently from the group consisting of O, S, Se, NH and NR;

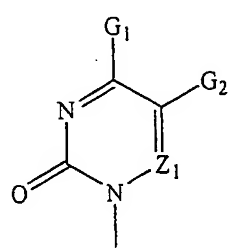
X^5 and X^6 are selected independently from the group consisting of O, S, Se, O_2, CY_2CO, CHOH, $C(OH)_2$, CH_2O, CH_2CH_2, CH_2CHNH_2, $CH_2CH_2CHNH_2$, CY_2OCY_2, CY_2, CRY, CY_2CY_2, CHR, CC, HC=CH, NH, NR, NOH, NOR, NNH_2, and NNHR;

X^7, X^8, X^9, and X^{10} are selected independently from the group consisting of H, F, OH, SH, NH_2, NHOH, NHOR, $NHNH_2$, NHNHR,CN, N_3, $^-BH_3M^+$, R, OR, SR, SeH, SeR, NHR, and NR_2.

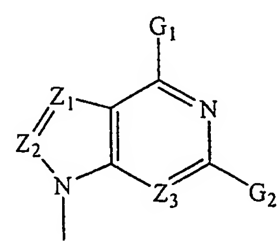
wherein n is 0 or 1;

R^1, R^2, $R^{2'}$, R^3, $R^{3'}$, and R^4 are selected independently from the group consisting of H, F, Cl, Br, OH, SH, NH_2, NHOH, N_3, NO_2, CHO, COOH, CN, $CONH_2$, COOR, R, OR, SR, SSR, NHR, and NR_2; alternatively, R^2 and $R^{2'}$ together and R^3 and $R^{3'}$ together independently are =O, =S, or =J-Q, where J is N, CH, CF, CCl, or CBr and Q is H, F, Cl, Br, N_3, or R;

D is a nucleoside base of Formula (**III**) or (**IV**):



(III)



(IV)

Z^1, Z^2, and Z^3 are independently N, CH or C-G^3;

G^1, G^2, and G^3 are selected independently from the group consisting of H, F, Cl, Br, I, OH, SH, NH_2, NHOH, $NHNH_2$, N_3, NO, NO_2, CHO, COOH, CN, $CONH_2$, CONHR, $C(S)NH_2$, C(S)NHR, COOR, R, OR, SR, NHR, and NR_2; when two of G^3 are present on a molecule they may be same as or different from each other; and

R is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, acyl, and aralkyl each optionally containing one or more heteroatoms;

with provisos that:

(1) when L is CH_2O and is linked to P through O and if one or more of X^7-X^{10} is selected from SH, F, SR, R, OR, NH_2, NHR, and NR_2, then X^2-X^6 and the rest of X^7-X^{10} are not all O or OH;

(2) when L is CH_2O and is linked to P through O and X^7- X^{10} are OH or OR, not all X^2-X^4 are O;

(3) when L is CH_2O and is linked to P through O and if one of X^2-X^4 is selected from S, NH, NR, NH, then X^5-X^{10} are not all O or OH, and one of X^5-X^{10} cannot be S when the rest of X^5-X^{10} are O or OH;

(4) when L is CH_2O and is linked to P through O and if the following X^6-X^5 pairs are CY_2-O, CH_2O-O, CH_2-S, CHMe-O, $CHCH_2NH_2$, CO-O, CHOH-O, $C(OH)_2$-O, NH-O, NH-S, NMe-O, NMe-NMe, Naryl-O, NH-NH, CH_2-CH_2, CF_2-CF_2, OCH_2O-O, O-CH_2, O-CF_2, O-NH, O-NMe, O-S, S-O, OO-O, O-SS, CC-O, C=C-O, or O_2-O then the X^2-X^4 and X^7-X^{10} are not all O or OH, and X^7-X^{10} is not one or more F or SEt when X^2-X^4 and the rest of X^7-X^{10} are O or OH;

(5) when L is CH_2O and is linked to P through O and if a X^2=P-X^7 is selected from $O{=}P(O)_2BH_3^-$, $O{=}P(O)_2NHR$, $S{=}P(O)_2BH_3$, $Se{=}P(O)_2OH$, $S{=}P(O)_2SH$, $S{=}P(O)_2NHR$, and $Se{=}P(O)_2SH$, at least one of X^3-X^6, X^8-X^{10} is not O or OH;

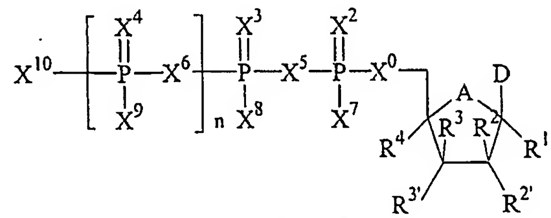
(6) when L is CH_2O or CH_2S and is linked to P through O or S respectively, one or more of X^2-X^{10} cannot be S;

(7) when L is CH_2O and is linked to P through O and one of X^7-X^{10} is SH, then X^2-X^6 and the rest of X^7-X^{10} are not all O or OH;

(8) when L is CH_2O and is linked to P through O and if the following X^6-X^5 pairs are CH_2-O, O-CH_2, NH-O, or CH_2-CH_2, then at least one of X^2-X^4 is not O or S; or at least of one of X^7-X^{10} is not O, S, or Me; and

(9) when L is CH_2O and linked to P through CH_2 and if A is O or CH_2 and X^{10} is alkyl, aryl, alkoxy, aryloxy, alkylamino, arylamino, or glyceroloxy, X^6 is not methylene and substituted methylene.

36. The compound defined in claim 35 of Formula **(VI)**:



(VI)

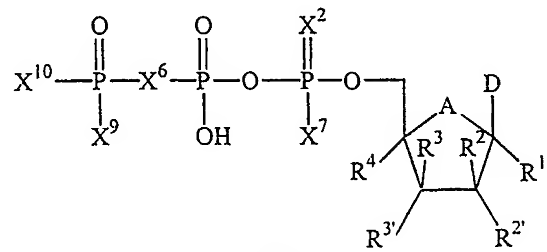
wherein X^0 is O, S, or NH;

wherein X^2, X^3, and X^4 are O or S;

wherein X^5 and X^6 are selected independently from the group consisting of O, S, NH, NR, CY_2;

wherein X^7, X^8, X^9, and X^{10} are selected independently from the group consisting of H, F, OH, SH, NH_2, NHOH, $^-BH_3M^+$, R, OR, SR, and NHR.

37. The compound defined in claim 35 of Formula **(VII)**:



(VII)

wherein X^2 is O or S;

wherein X^6 is NH or CY_2;

wherein X^7 is selected from the group consisting of H, F, SH, NH_2, NHOH, $^-BH_3M^+$, R, SR, and NHR; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, NH_2, NHOH, $^-BH_3M^+$, R, OR, SR, and NHR.

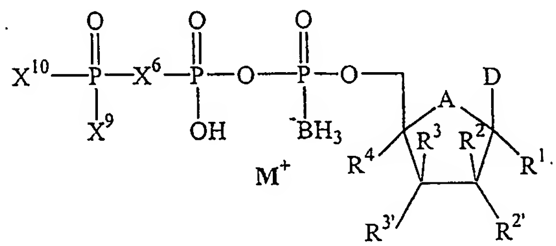
38. The compound defined in claim 37

wherein X^2 is O; and

wherein X^7 is selected from the group consisting of SH, NH_2, $^{-}BH_3M^+$, alkyl, aryl, alkylamino, and arylamino.

39. The compound defined in claim 37
wherein X^2 is O; and
wherein X^7 is selected from the group consisting of SH, NH_2, $^{-}BH_3M^+$, R, and NHR.

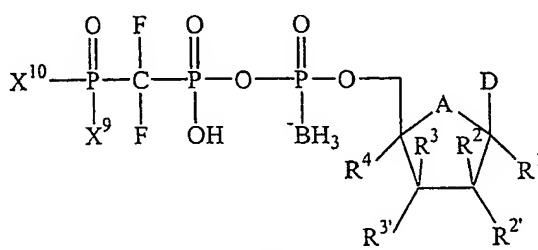
40. The compound defined in claim 35 of Formula **(VIII)**:



(VIII)

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, alkyl, alkoxy, aryl, and aryloxy.

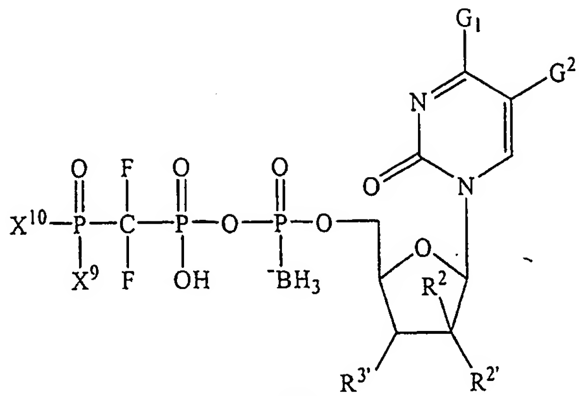
41. The compound defined in claim 35 of Formula **(IX)**:



(IX)

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy and aryloxy.

42. The compound defined in claim 35 of Formula **(X)**:



(X)

wherein R^2 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

wherein $R^{2'}$ is H, F, OH, or OMe, alkyl, methoxyethoxy, or NH_2;

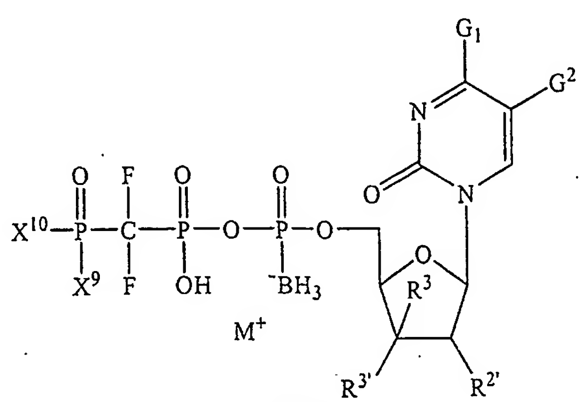
wherein $R^{3'}$ is H, F, OH, or N_3;

wherein G^1 is OH or NH_2;

wherein G^2 is H or Me; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy, and aryloxy.

43. The compound defined in claim 35 of Formula (XI):



(XI)

wherein $R^{2'}$ is H, F, OH, or OMe;

wherein R^3 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

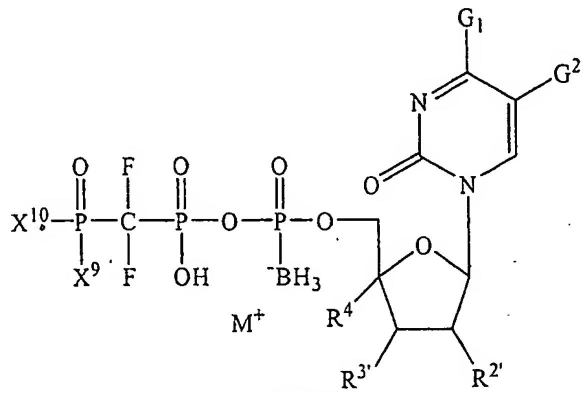
wherein $R^{3'}$ is H, F, OH, or N_3;

wherein G^1 is OH or NH_2;

wherein G^2 is H or Me; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy, and aryloxy.

44. The compound defined in claim 35 of Formula **(XII)**:



(XII)

wherein $R^{2'}$ is H, F, OH, or OMe;

wherein $R^{3'}$ is H, F, OH, or N_3;

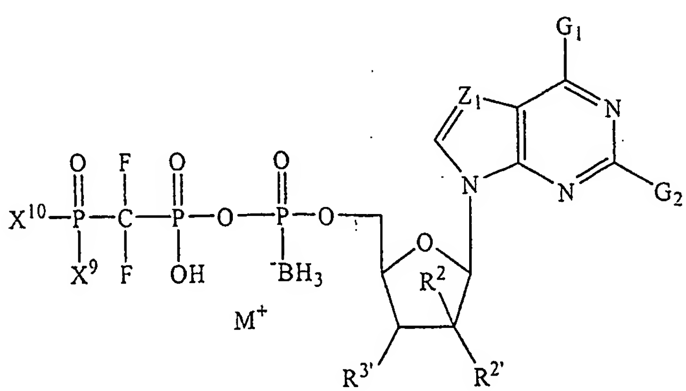
wherein R^4 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

wherein G^1 is OH or NH_2;

wherein G^2 is H or Me; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy, and aryloxy.

45. The compound defined in claim 35 of Formula **(XIII)**:



(XIII)

wherein R^2 is methyl, ethyl, vinyl, ethynyl, hydroxymethyl, or haloalkyl;

wherein $R^{2'}$ is H, F, OH, OCH_3, or methoxyethoxy;

wherein $R^{3'}$ is H, F, OH, N_3, NH_2, or CH_2OH;

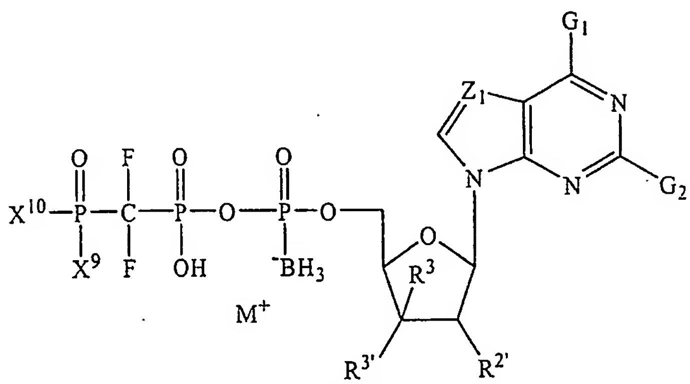
wherein G^1 is OH, NH_2, Cl, OMe, NH-cyclopropyl, SH, or S-alkyl;

wherein G^2 is H, NH_2, NHR, F, Cl, Br, or I;

wherein Z^1 is N or CH; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy and aryloxy.

46. The compound defined in claim 35 of Formula **(XIV)**:



(XIV)

wherein $R^{2'}$ is H, F, OH or OCH_3;

wherein R^3 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

wherein $R^{3'}$ is H, F, OH, or N_3;

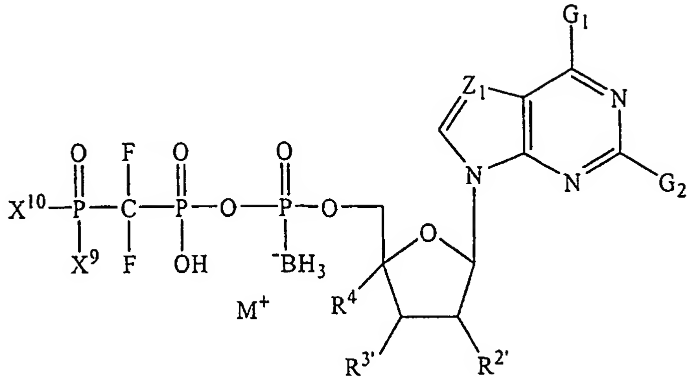
wherein G^1 is OH or NH_2;

wherein G^2 is H or NH_2;

wherein Z^1 is N or CH; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy and aryloxy.

47. The compound defined in claim 35 of Formula (XV):



(XV)

wherein $R^{2'}$ is H, F, OH or OCH_3;

wherein $R^{3'}$ is H, F, OH, or N_3;

wherein R^4 is methyl, ethyl, vinyl, ethynyl, or hydroxymethyl;

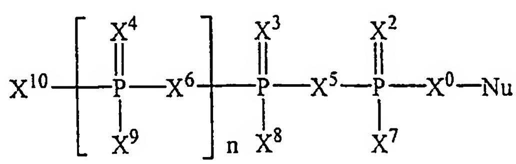
wherein G^1 is OH or NH_2;

wherein G^2 is H or NH_2;

wherein Z^1 is N or CH; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, alkoxy and aryloxy.

48. A compound of Formula (XVI):



(XVI)

wherein X^0, X^5 and X^6 are selected independently from the group consisting of O, S, NH, CY_2;

wherein X^2, X^3, and X^4 are each independently O, S, or Se;

wherein X^7, X^8, X^9, and X^{10} are selected independently from the group consisting of H, F, OH, SH, NH_2, NHOH, CN, N_3, $^-BH_3M^+$, R, OR, SR, SeH, SeR, NHR, and NR_2;

wherein n is 0 or 1;

wherein Nu is selected from the group consisting of natural nucleosides, sugar-modified nucleosides, base-modified nucleosides, and nucleosides with both sugar and base modifications;

wherein Nu is linked to X^0 through CH_2 of the sugar moiety of Nu; and

R is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, acyl, and aralkyl each optionally containing one or more heteroatoms;

with provisos that:

(1) when L is CH_2O and is linked to P through O and if one or more of X^7-X^{10} is selected from SH, F, SR, R, OR, NH_2, NHR, and NR_2, then X^2-X^6 and the rest of X^7-X^{10} are not all O or OH;

(2) when L is CH_2O and is linked to P through O and X^7- X^{10} are OH or OR, not all X^2-X^4 are O;

(3) when L is CH_2O and is linked to P through O and if one of X^2-X^4 is selected from S, NH, NR, NH, then X^5-X^{10} are not all O or OH, and one of X^5-X^{10} cannot be S when the rest of X^5-X^{10} are O or OH;

(4) when L is CH_2O and is linked to P through O and if the following X^6-X^5 pairs are CY_2-O, CH_2O-O, CH_2-S, CHMe-O, $CHCH_2NH_2$, CO-O, CHOH-O, $C(OH)_2$-O, NH-O, NH-S, NMe-O, NMe-NMe, Naryl-O, NH-NH, CH_2-CH_2, CF_2-CF_2, OCH_2O-O, O-CH_2, O-CF_2, O-NH, O-NMe, O-S, S-O, OO-O, O-SS, CC-O, C=C-O, or O_2-O then the X^2-X^4 and X^7-X^{10} are not all O or OH, and X^7-X^{10} is not one or more F or SEt when X^2-X^4 and the rest of X^7-X^{10} are O or OH;

(5) when L is CH_2O and is linked to P through O and if a X^2=P-X^7 is selected from $O{=}P(O)_2BH_3^-$, $O{=}P(O)_2NHR$, $S{=}P(O)_2BH_3$, $Se{=}P(O)_2OH$, $S{=}P(O)_2SH$, $S{=}P(O)_2NHR$, and $Se{=}P(O)_2SH$, at least one of X^3-X^6, X^8-X^{10} is not O or OH;

(6) when L is CH_2O or CH_2S and is linked to P through O or S respectively, one or more of X^2-X^{10} cannot be S;

(7) when L is CH_2O and is linked to P through O and one of X^7-X^{10} is SH, then X^2-X^6 and the rest of X^7-X^{10} are not all O or OH;

(8) when L is CH_2O and is linked to P through O and if the following X^6-X^5 pairs are CH_2-O, O-CH_2, NH-O, or CH_2-CH_2, then at least one of X^2-X^4 is not O or S; or at least of one of X^7-X^{10} is not O, S, or Me; and

(9) when L is CH_2O and linked to P through CH_2 and if A is O or CH_2 and X^{10} is alkyl, aryl, alkoxy, aryloxy, alkylamino, arylamino, or glyceroloxy, X^6 is not methylene and substituted methylene.

49. The compound defined in claim 48 where Nu is selected from the group consisting of:

adenosine,
cytidine,
guanosine,
uridine,
2'-deoxyadenosine,
2'-deoxycytidine,
2'-deoxyguanosine,
thymidine,
inosine,

9-(β–D-arabinofuranosyl)adenine,
1-(β-D-arabinofuranosyl)cytosine,
9-(β-D-arabinofuranosyl)guanine,
1-(β-D-arabinofuranosyl)uracil,
9-(β–D-arabinofuranosyl)hypoxanthine,
1-(β-D-arabinofuranosyl)thymine,

3'-azido-3'-deoxythymidine,
3'-azido-2',3'-dideoxyuridine,
3'-azido-2',3'-dideoxycytidine,
3'-azido-2',3'-dideoxyadenosine,
3'-azido-2',3'-dideoxyguanosine,
3'-azido-2',3'-dideoxyinosine,

3'-deoxythymidine,
2',3'-dideoxyuridine,
2',3'-dideoxyinosine,
2',3'-dideoxyadenosine,
2',3'-dideoxycytidine,
2',3'-dideoxyguanosine,
9-(2,3-dideoxy-1-β-D-ribofuranosyl)-2,6-diaminopurine,

3'-deoxy-2',3'-didehydrothymidine,
2',3'-didehydro-2',3'-dideoxyuridine,
2',3'-didehydro-2',3'-dideoxycytidine,
2',3'-didehydro-2',3'-dideoxyadenosine,
2',3'-didehydro-2',3'-dideoxyguanosine,
2',3'-didehydro-2',3'-dideoxyinosine,

3-deazaadenosine,
3-deazaguanosine,
3-deazainosine,
7-deazaadenosine,
7-deazaguanosine,
7-deazainosine,

6-azauridine,
6-azathymidine,
6-azacytidine,
5-azacytidine,

9-(β-D-ribofuranosyl)-6-thiopurine,
6-methylthio-9-(β-D-ribofuranosyl)purine,
2-amino-9-(β-D-ribofuranosyl)-6-thiopurine,
2-amino-6-methylthio-9-(β-D-ribofuranosyl)purine,

5-fluorocytidine,
5-iodocytidine,
5-bromocytidine,
5-chlorocytidine,
5-fluorouridine,
5-iodouridine,
5-bromouridine,
5-chlorouridine,

2'-*C*-methyladenosine,
2'-*C*-methylcytidine,
2'-*C*-methylguanosine,
2'-*C*-methylinosine,
2'-*C*-methyluridine,
2'-*C*-methylthymidine,

2'-deoxy-2'-fluoroadenosine,
2'-deoxy-2'-fluorocytidine,
2'-deoxy-2'-fluoroguanosine,
2'-deoxy-2'-fluorouridine,
2'-deoxy-2'-fluoroinosine,
2'-α-fluorothymidine,

2'-deoxy-2'-fluoroarabinoadenosine,
2'-deoxy-2'-fluoroarabinocytidine,
2'-deoxy-2'-fluoroarabinoguanosine,
2'-deoxy-2'-fluoroarabinouridine,
2'-deoxy-2'-fluoroarabinoinosine,
2'-β-fluorothymidine,

2'-*O*-methyladenosine,
2'-*O*-methylcytidine,
2'-*O*-methylguanosine,
2'-*O*-methylinosine,
2'-*O*-5-dimethyluridine,

2'-*C*-ethynylcytidine,
2'-*C*-ethynylguanosine,
2'-*C*-ethynyluridine,
2'-*C*-ethynylinosine,
2'-*C*-ethynyl-5-methyluridine,

3'-*C*-ethynyladenosine,
3'-*C*-ethynylcytidine,
3'-*C*-ethynylguanosine,
3'-*C*-ethynyluridine,
3'-*C*-ethynylinosine,
3'-*C*-ethynyl-5-methyluridine,

3'-deoxyadenosine,
3'-deoxycytidine,
3'-deoxyguanosine,
3'-deoxyuridine,
3'-deoxyinosine,

4'-*C*-ethynyladenosine,
4'-*C*-ethynylcytidine,
4'-*C*-ethynylguanosine,
4'-*C*-ethynyluridine,
4'-*C*-ethynylinosine,
4'-*C*-ethynylthymidine,

4'-*C*-methyladenosine,
4'-*C*-methylcytidine,
4'-*C*-methylguanosine,
4'-*C*-methyluridine,
4'-*C*-methylinosine,
4'-*C*-methylthymidine,

2'-*C*-methyl-7-deazaadenosine,

2'-*C*-methyl-7-deazaguanosine,
2'-*C*-methyl-3-deazaadenosine,
2'-*C*-methyl-3-deazaguanosine,
2'-*O*-methyl-7-deazaadenosine,
2'-*O*-methyl-7-deazaguanosine,
2'-*O*-methyl-3-deazaadenosine,
2'-*O*-methyl-3-deazaguanosine,
2'-*C*-methyl-6-azauridine,
2'-*C*-methyl-5-fluorouridine,
2'-*C*-methyl-5-fluorocytidine,
2'-*C*-methyl-2-chloroadenosine,

2'-deoxy-7-deazaadenosine,
2'-deoxy-3-deazaadenosine,
2'-deoxy-7-deazaguanosine,
2'-deoxy-3-deazaguanosine,
2'-deoxy-6-azauridine,
2'-deoxy-5-fluorouridine,
2'-deoxy-5-fluorocytidine,
2'-deoxy-5-iodouridine,
2'-deoxy-5-iodocytidine,
2'-deoxy-2-chloroadenosine,
2'-deoxy-2-fluoroadenosine,

3'-deoxy-7-deazaadenosine,
3'-deoxy-7-deazaguanosine,
3'-deoxy-3-deazaadenosine,
3'-deoxy-3-deazaguanosine,
3'-deoxy-6-azauridine,
3'-deoxy-5-fluorouridine,
3'-deoxy-5-iodouridine,
3'-deoxy-5-fluorocytidine,
3'-deoxy-2-chloroadenosine,

2',3'-dideoxy-7-deazaadenosine,
2',3'-dideoxy-7-deazaguanosine,
2',3'-dideoxy-3-deazaadenosine,
2',3'-dideoxy-3-deazaguanosine,
2',3'-dideoxy-6-azauridine,
2',3'-dideoxy-5-fluorouridine,
2',3'-dideoxy-5-fluorouridine,
2',3'-dideoxy-5-iodocytidine,
2',3'-dideoxy-2-chloroadenosine,

2',3'-dideoxy-β-L-cytidine,
2',3'-dideoxy-β-L-adenosine,
2',3'-dideoxy-β-L-guanosine,

3'-deoxy-β-L-thymidine,
2',3'-dideoxy-5-fluoro-β-L-cytidine,

β-L-thymidine,
2'-deoxy-β-L-cytidine,
2'-deoxy-β-L-adenosine,
2'-deoxy-β-L-guanosine,
2'-deoxy-β-L-inosine,

β-L-cytidine,
β-L-adenosine,
β-L-guanosine,
β-L-uridine,
β-L-inosine,

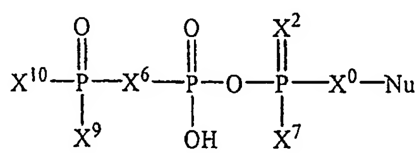
2',3'-didehydro-2',3'-dideoxy-β-L-cytidine,
2',3'-didehydro-3'-dideoxy-β-L-thymidine,
2',3'-didehydro-2',3'-dideoxy-β-L-adenosine,
2',3'-didehydro-2',3'-dideoxy-β-L-guanosine,
2',3'-didehydro-2',3'-dideoxy-β-L-5-fluorocytidine,

2'-deoxy-2',2'-difluorocytidine,
9-(β-D-arabinofuranosyl)-2-fluoroadenine,
2'-deoxy-2'(E)-fluoromethylenecytidine,
2'-deoxy-2'(Z)-fluoromethylenecytidine,
(-)-2',3'-dideoxy-3'-thiacytidine,
(+)-2',3'-dideoxy-3'-thiacytidine,

1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-D-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-L-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-D-ribofuranosyl-5-ethynylimidazole-4-carboxamide,
1-β-L-ribofuranosyl-5-ethynylimidazole-4-carboxamide,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine,
1-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)-5-methyluracil,
1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil,
E-5-(2-bromovinyl)-2'-deoxyuridine,
5-trifluoromethylthymidine,
1-β-D-arabinofuranosyl-5-propynyluracil,
1-(2-deoxy-2-fluoro-1-β-D-arabinofuranosyl)-5-ethyluracil,
2',3'-dideoxy-3'-fluoroguanosine,
3'-deoxy-3'-fluorothymidine,

(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(1*R*,3*S*,4*R*)-9-(3-hydroxy-4-hydroxymethylcyclopent-1-yl)guanine,
(1*S*,2*R*,4*R*)-9-(1-hydroxy-2-hydroxymethylcyclopent-4-yl)guanine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)-2,6-diaminopurine,
(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)cytosine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)guanine,
(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)-5-fluorocytosine,
(1*R*,2*S*,4*S*)-9-(4-hydroxy-3-hydroxymethyl-2-methylenecyclopent-4-yl]guanine, and
(1*S*,3*R*,4*S*)-9-(3-hydroxy-4-hydroxymethyl-5-methylenecyclopent-1-yl]guanine.

50. The compound defined in claim 48 of Formula **(XVII)**:



(XVII)

wherein X^0 and X^6 are selected independently from the group consisting of O, S, NH, CY_2;

wherein X^2 is O or S;

wherein X^7 is selected from the group consisting of OH, F, SH, NH_2, NHOH $^-BH_3M^+$, R, SR, and NHR; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, NH_2, NHOH, $^-BH_3M^+$, R, OR, SR and NHR.

51. The compound defined in claim 50 where Nu is selected from the group consisting of:

adenosine,
cytidine,
guanosine,
uridine,
2'-deoxyadenosine,
2'-deoxycytidine,
2'-deoxyguanosine,
thymidine,
inosine,

9-(β–D-arabinofuranosyl)adenine,
1-(β-D-arabinofuranosyl)cytosine,
9-(β-D-arabinofuranosyl)guanine,
1-(β-D-arabinofuranosyl)uracil,
9-(β–D-arabinofuranosyl)hypoxanthine,
1-(β-D-arabinofuranosyl)thymine,

3'-azido-3'-deoxythymidine,
3'-azido-2',3'-dideoxyuridine,
3'-azido-2',3'-dideoxycytidine,
3'-azido-2',3'-dideoxyadenosine,
3'-azido-2',3'-dideoxyguanosine,
3'-azido-2',3'-dideoxyinosine,

3'-deoxythymidine,
2',3'-dideoxyuridine,
2',3'-dideoxyinosine,
2',3'-dideoxyadenosine,
2',3'-dideoxycytidine,
2',3'-dideoxyguanosine,
9-(2,3-dideoxy-1-β-D-ribofuranosyl)-2,6-diaminopurine,

3'-deoxy-2',3'-didehydrothymidine,
2',3'-didehydro-2',3'-dideoxyuridine,
2',3'-didehydro-2',3'-dideoxycytidine,
2',3'-didehydro-2',3'-dideoxyadenosine,
2',3'-didehydro-2',3'-dideoxyguanosine,
2',3'-didehydro-2',3'-dideoxyinosine,

3-deazaadenosine,
3-deazaguanosine,
3-deazainosine,
7-deazaadenosine,
7-deazaguanosine,

7-deazainosine,

6-azauridine,
6-azathymidine,
6-azacytidine,
5-azacytidine,

9-(β-D-ribofuranosyl)-6-thiopurine,
6-methylthio-9-(β-D-ribofuranosyl)purine,
2-amino-9-(β-D-ribofuranosyl)-6-thiopurine,
2-amino-6-methylthio-9-(β-D-ribofuranosyl)purine,

5-fluorocytidine,
5-iodocytidine,
5-bromocytidine,
5-chlorocytidine,
5-fluorouridine,
5-iodouridine,
5-bromouridine,
5-chlorouridine,

2'-*C*-methyladenosine,
2'-*C*-methylcytidine,
2'-*C*-methylguanosine,
2'-*C*-methylinosine,
2'-*C*-methyluridine,
2'-*C*-methylthymidine,

2'-deoxy-2'-fluoroadenosine,
2'-deoxy-2'-fluorocytidine,
2'-deoxy-2'-fluoroguanosine,
2'-deoxy-2'-fluorouridine,
2'-deoxy-2'-fluoroinosine,
2'-α-fluorothymidine,

2'-deoxy-2'-fluoroarabinoadenosine,
2'-deoxy-2'-fluoroarabinocytidine,
2'-deoxy-2'-fluoroarabinoguanosine,
2'-deoxy-2'-fluoroarabinouridine,
2'-deoxy-2'-fluoroarabinoinosine,
2'-β-fluorothymidine,

2'-*O*-methyladenosine,
2'-*O*-methylcytidine,
2'-*O*-methylguanosine,
2'-*O*-methylinosine,
2'-*O*-5-dimethyluridine,

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β-L-uridine,
β-L-inosine,

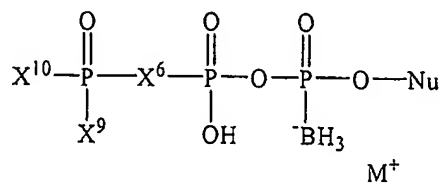
2',3'-didehydro-2',3'-dideoxy-β-L-cytidine,
2',3'-didehydro-3'-dideoxy-β-L-thymidine,
2',3'-didehydro-2',3'-dideoxy-β-L-adenosine,
2',3'-didehydro-2',3'-dideoxy-β-L-guanosine,
2',3'-didehydro-2',3'-dideoxy-β-L-5-fluorocytidine,

2'-deoxy-2',2'-difluorocytidine,
9-(β-D-arabinofuranosyl)-2-fluoroadenine,
2'-deoxy-2'(E)-fluoromethylenecytidine,
2'-deoxy-2'(Z)-fluoromethylenecytidine,
(-)-2',3'-dideoxy-3'-thiacytidine,
(+)-2',3'-dideoxy-3'-thiacytidine,

1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-D-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-L-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-D-ribofuranosyl-5-ethynylimidazole-4-carboxamide,
1-β-L-ribofuranosyl-5-ethynylimidazole-4-carboxamide,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine,
1-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)-5-methyluracil,
1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil,
E-5-(2-bromovinyl)-2'-deoxyuridine,
5-trifluoromethylthymidine,
1-β-D-arabinofuranosyl-5-propynyluracil,
1-(2-deoxy-2-fluoro-1-β-D-arabinofuranosyl)-5-ethyluracil,
2',3'-dideoxy-3'-fluoroguanosine,
3'-deoxy-3'-fluorothymidine,

(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(1*R*,3*S*,4*R*)-9-(3-hydroxy-4-hydroxymethylcyclopent-1-yl)guanine,
(1*S*,2*R*,4*R*)-9-(1-hydroxy-2-hydroxymethylcyclopent-4-yl)guanine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)-2,6-diaminopurine,
(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)cytosine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)guanine,
(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)-5-fluorocytosine,
(1*R*,2*S*,4*S*)-9-(4-hydroxy-3-hydroxymethyl-2-methylenecyclopent-4-yl]guanine, and
(1*S*,3*R*,4*S*)-9-(3-hydroxy-4-hydroxymethyl-5-methylenecyclopent-1-yl]guanine.

52. The compound defined in claim 48 of Formula **(XVIII)**:



(XVIII)

wherein X^6 is NH or CY_2; and

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, alkyl, alkoxy, aryl and aryloxy.

53. The compound defined in claim 52 where Nu is selected from the group consisting of:

adenosine,
cytidine,
guanosine,
uridine,
2'-deoxyadenosine,
2'-deoxycytidine,
2'-deoxyguanosine,
thymidine,
inosine,

9-(β–D-arabinofuranosyl)adenine,
1-(β-D-arabinofuranosyl)cytosine,
9-(β-D-arabinofuranosyl)guanine,
1-(β-D-arabinofuranosyl)uracil,
9-(β–D-arabinofuranosyl)hypoxanthine,
1-(β-D-arabinofuranosyl)thymine,

3'-azido-3'-deoxythymidine,
3'-azido-2',3'-dideoxyuridine,
3'-azido-2',3'-dideoxycytidine,
3'-azido-2',3'-dideoxyadenosine,
3'-azido-2',3'-dideoxyguanosine,
3'-azido-2',3'-dideoxyinosine,

3'-deoxythymidine,
2',3'-dideoxyuridine,
2',3'-dideoxyinosine,
2',3'-dideoxyadenosine,

2',3'-dideoxycytidine,
2',3'-dideoxyguanosine,
9-(2,3-dideoxy-1-β-D-ribofuranosyl)-2,6-diaminopurine,

3'-deoxy-2',3'-didehydrothymidine,
2',3'-didehydro-2',3'-dideoxyuridine,
2',3'-didehydro-2',3'-dideoxycytidine,
2',3'-didehydro-2',3'-dideoxyadenosine,
2',3'-didehydro-2',3'-dideoxyguanosine,
2',3'-didehydro-2',3'-dideoxyinosine,

3-deazaadenosine,
3-deazaguanosine,
3-deazainosine,
7-deazaadenosine,
7-deazaguanosine,
7-deazainosine,

6-azauridine,
6-azathymidine,
6-azacytidine,
5-azacytidine,

9-(β-D-ribofuranosyl)-6-thiopurine,
6-methylthio-9-(β-D-ribofuranosyl)purine,
2-amino-9-(β-D-ribofuranosyl)-6-thiopurine,
2-amino-6-methylthio-9-(β-D-ribofuranosyl)purine,

5-fluorocytidine,
5-iodocytidine,
5-bromocytidine,
5-chlorocytidine,
5-fluorouridine,
5-iodouridine,
5-bromouridine,
5-chlorouridine,

2'-*C*-methyladenosine,
2'-*C*-methylcytidine,
2'-*C*-methylguanosine,
2'-*C*-methylinosine,
2'-*C*-methyluridine,
2'-*C*-methylthymidine,

2'-deoxy-2'-fluoroadenosine,
2'-deoxy-2'-fluorocytidine,
2'-deoxy-2'-fluoroguanosine,

2'-deoxy-2'-fluorouridine,
2'-deoxy-2'-fluoroinosine,
2'-α-fluorothymidine,

2'-deoxy-2'-fluoroarabinoadenosine,
2'-deoxy-2'-fluoroarabinocytidine,
2'-deoxy-2'-fluoroarabinoguanosine,
2'-deoxy-2'-fluoroarabinouridine,
2'-deoxy-2'-fluoroarabinoinosine,
2'-β-fluorothymidine,

2'-*O*-methyladenosine,
2'-*O*-methylcytidine,
2'-*O*-methylguanosine,
2'-*O*-methylinosine,
2'-*O*-5-dimethyluridine,

2'-*C*-ethynylcytidine,
2'-*C*-ethynylguanosine,
2'-*C*-ethynyluridine,
2'-*C*-ethynylinosine,
2'-*C*-ethynyl-5-methyluridine,

3'-*C*-ethynyladenosine,
3'-*C*-ethynylcytidine,
3'-*C*-ethynylguanosine,
3'-*C*-ethynyluridine,
3'-*C*-ethynylinosine,
3'-*C*-ethynyl-5-methyluridine,

3'-deoxyadenosine,
3'-deoxycytidine,
3'-deoxyguanosine,
3'-deoxyuridine,
3'-deoxyinosine,

4'-*C*-ethynyladenosine,
4'-*C*-ethynylcytidine,
4'-*C*-ethynylguanosine,
4'-*C*-ethynyluridine,
4'-*C*-ethynylinosine,
4'-*C*-ethynylthymidine,

4'-*C*-methyladenosine,
4'-*C*-methylcytidine,
4'-*C*-methylguanosine,
4'-*C*-methyluridine,

4'-*C*-methylinosine,
4'-*C*-methylthymidine,

2'-*C*-methyl-7-deazaadenosine,
2'-*C*-methyl-7-deazaguanosine,
2'-*C*-methyl-3-deazaadenosine,
2'-*C*-methyl-3-deazaguanosine,
2'-*O*-methyl-7-deazaadenosine,
2'-*O*-methyl-7-deazaguanosine,
2'-*O*-methyl-3-deazaadenosine,
2'-*O*-methyl-3-deazaguanosine,
2'-*C*-methyl-6-azauridine,
2'-*C*-methyl-5-fluorouridine,
2'-*C*-methyl-5-fluorocytidine,
2'-*C*-methyl-2-chloroadenosine,

2'-deoxy-7-deazaadenosine,
2'-deoxy-3-deazaadenosine,
2'-deoxy-7-deazaguanosine,
2'-deoxy-3-deazaguanosine,
2'-deoxy-6-azauridine,
2'-deoxy-5-fluorouridine,
2'-deoxy-5-fluorocytidine,
2'-deoxy-5-iodouridine,
2'-deoxy-5-iodocytidine,
2'-deoxy-2-chloroadenosine,
2'-deoxy-2-fluoroadenosine,

3'-deoxy-7-deazaadenosine,
3'-deoxy-7-deazaguanosine,
3'-deoxy-3-deazaadenosine,
3'-deoxy-3-deazaguanosine,
3'-deoxy-6-azauridine,
3'-deoxy-5-fluorouridine,
3'-deoxy-5-iodouridine,
3'-deoxy-5-fluorocytidine,
3'-deoxy-2-chloroadenosine,

2',3'-dideoxy-7-deazaadenosine,
2',3'-dideoxy-7-deazaguanosine,
2',3'-dideoxy-3-deazaadenosine,
2',3'-dideoxy-3-deazaguanosine,
2',3'-dideoxy-6-azauridine,
2',3'-dideoxy-5-fluorouridine,
2',3'-dideoxy-5-fluorouridine,
2',3'-dideoxy-5-iodocytidine,
2',3'-dideoxy-2-chloroadenosine,

2',3'-dideoxy-β-L-cytidine,
2',3'-dideoxy-β-L-adenosine,
2',3'-dideoxy-β-L-guanosine,
3'-deoxy-β-L-thymidine,
2',3'-dideoxy-5-fluoro-β-L-cytidine,

β-L-thymidine,
2'-deoxy-β-L-cytidine,
2'-deoxy-β-L-adenosine,
2'-deoxy-β-L-guanosine,
2'-deoxy-β-L-inosine,

β-L-cytidine,
β-L-adenosine,
β-L-guanosine,
β-L-uridine,
β-L-inosine,

2',3'-didehydro-2',3'-dideoxy-β-L-cytidine,
2',3'-didehydro-3'-dideoxy-β-L-thymidine,
2',3'-didehydro-2',3'-dideoxy-β-L-adenosine,
2',3'-didehydro-2',3'-dideoxy-β-L-guanosine,
2',3'-didehydro-2',3'-dideoxy-β-L-5-fluorocytidine,

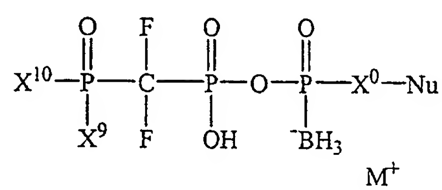
2'-deoxy-2',2'-difluorocytidine,
9-(β-D-arabinofuranosyl)-2-fluoroadenine,
2'-deoxy-2'(E)-fluoromethylenecytidine,
2'-deoxy-2'(Z)-fluoromethylenecytidine,
(-)-2',3'-dideoxy-3'-thiacytidine,
(+)-2',3'-dideoxy-3'-thiacytidine,

1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide,
1-β-D-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-L-ribofuranosyl-1,3-imidazolium-5-olate,
1-β-D-ribofuranosyl-5-ethynylimidazole-4-carboxamide,
1-β-L-ribofuranosyl-5-ethynylimidazole-4-carboxamide,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil,
1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine,
1-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)-5-methyluracil,
1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil,
E-5-(2-bromovinyl)-2'-deoxyuridine,
5-trifluoromethylthymidine,
1-β-D-arabinofuranosyl-5-propynyluracil,

1-(2-deoxy-2-fluoro-1-β-D-arabinofuranosyl)-5-ethyluracil,
2',3'-dideoxy-3'-fluoroguanosine,
3'-deoxy-3'-fluorothymidine,

(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine,
(±)-(1β,2α,3β)-9-[2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine,
(1*R*,3*S*,4*R*)-9-(3-hydroxy-4-hydroxymethylcyclopent-1-yl)guanine,
(1*S*,2*R*,4*R*)-9-(1-hydroxy-2-hydroxymethylcyclopent-4-yl)guanine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)-2,6-diaminopurine,
(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)cytosine,
(2*R*,4*R*)-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)guanine,
(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)-5-fluorocytosine,
(1*R*,2*S*,4*S*)-9-(4-hydroxy-3-hydroxymethyl-2-methylenecyclopent-4-yl]guanine, and
(1*S*,3*R*,4*S*)-9-(3-hydroxy-4-hydroxymethyl-5-methylenecyclopent-1-yl]guanine.

54. The compound defined in claim 48 Formula **(XIX)**:



(XIX)

wherein X^9 and X^{10} are selected independently from the group consisting of OH, SH, alkyl, alkoxy, aryl and aryloxy.

55. A pharmaceutical composition comprising a therapeutically effective amount of the compound according to any of claims 1-34, a pharmaceutically acceptable salt thereof, optionally in combination with one or more other active ingredients and/or with a pharmaceutically acceptable carrier

56. A pharmaceutical composition comprising a therapeutically effective amount of the compound according to any of claims 35-54, a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable prodrug thereof, optionally in combination with one or more other active ingredients and/or with a pharmaceutically acceptable carrier

57. A method for the treatment of a microbial infection comprising administering a therapeutically effective amount of the compound defined in any of claims 1-54.

58. A method for the treatment of a proliferative disorder comprising administering a therapeutically effective amount of the compound defined in any of claims 1-54.